The role of iron in the pathogenesis of trichinellosis

by

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Go thou. I'll fetch some flax and
whites of eggs
To apply to his bleeding face. Now
heaven help him!

Shakespeare's King Lear, Act III,
Scene VII, Row 106-107; 1607.

... the ability of raw egg white to
combine with iron and make it unavail-
able to microorganisms for growth may
have significance for ... therapeutic
purposes ...

Schade L. Arthur and Caroline Leona: in
Science, Vol. 100, p. 15; 1944.

I. INTRODUCTION

In the struggle for existence, all of nature's beings try to fulfill their biological purpose, namely perpetuation of the species. Energy, a requisite for every biological function in the form of nutrients, is the object of competition among all organisms. This competition becomes more intense as available nutrients become limited. Every organism has some special means by which it tries to gain an advantage over its competitors in competition for the nutrients.

One such competition involves the parasitic way of life, in which one organism takes nutrients directly from another organism. Iron is one essential nutrient that many parasitic organisms acquire from their hosts by using special iron-assimilation mechanisms. The host, on the other hand, tries to make iron less available to its parasitic organisms. The outcome of this competition many times determines the fate of the parasitic organism or of the host.

In the past 40 years, it has been found that competition for iron between humans or animals and their parasitic microorganisms is of great importance for the pathogenesis of bacterial, fungal, and protozoan diseases (Weinberg, 1978). Specifically, it has been discovered that iron in the vertebrate hosts is not readily available to microorganisms because it is sequestered intracellularly or it is bound to certain proteins of the body fluids. These proteins are called iron-binding proteins (Finkelstein et al., 1983). The microorganisms, in order to obtain the necessary iron for their growth, produce powerful iron-binding

compounds of their own. Called siderophores, these iron-binding compounds compete for iron with the host's iron-binding proteins (Neilands, 1981). The ability of the microorganisms to produce siderophores is linked with their pathogenic virulence (Bullen et al., 1978). In an infection due to microorganisms, the host tries to make iron less available to the microorganisms by various mechanisms, one of which is the shift of iron from the plasma to reticuloendothelial system (RES), resulting in hypoferremia. On the other hand, in experimentally or naturally produced hyperferremia, the host is more susceptible to infections (Weinberg, 1978).

While a voluminous literature has developed on the role of iron in bacterial and fungal diseases since the first observation of Schade and Caroline in 1944, little is known about the role of iron in protozoan diseases, and even less in helminthic diseases.

In the present study, I will attempt to examine the role of iron in the pathogenesis of trichinellosis, an infection caused by the nematode Trichinella spiralis (Owen, 1835) Railliet, 1895 , by trying to answer the following questions:

a) Is iron important for the survival of T. spiralis?

b) Does the host react to trichinellosis with hypoferremia?

c) Does excess of iron in the host enhance the severity of trichinellosis?

Reasons for selecting T. spiralis as a model nematode were: availability, important health significance, the fact that it lives in an iron-rich environment, and the ease with which it can be handled.

II. LITERATURE REVIEW

A. Iron-Binding Compounds in Microbes

1. Importance of iron for microbial metabolism

The concentration of iron that is required for the growth of bacteria and fungi ranges from 0.4 to 4.0 μM (Weinberg, 1974). This trace amount of iron is absolutely necessary because iron is involved in many oxidation-reduction reactions due to its role as a co-factor in enzymic processes (Coughlan, 1971). The microbial enzymes in which iron is important are divided by Coughlan (1971) into four categories: a) the heme-enzymes, which are the cytochromes involved in electron and/or hydrogen transport, and the catalases and peroxidases involved in the breakdown of peroxide molecules, b) the oxygenases, which catalyze the incorporation of molecular oxygen in their substrates, c) the ferroflavoproteins, which catalyze the dehydrogenation of their substrates, and d) the ferredoxin and ferredoxin-like proteins, which are involved in redox systems like photosynthesis, nitrogen-fixation, electron transport and oxidative phosphorylation.

Coughlan (1971) has reviewed and summarized the consequences of iron-deficiency in microbes. The growth of many microbes is reduced or inhibited, and their structure may be altered. The oxidative metabolism of the microbes is reduced, and amounts of their metabolic products, such as porphyrins, antibiotics, specific pigments, toxins, vitamins and siderophores, may increase or decrease. Finally, the function of enzymes and

iron-containing proteins, as well as DNA formation, can be affected.

Since iron is so important for the life functions of microbes, as can be seen from the many disturbances that its absence causes to them, it is not surprising that microbes have evolved iron-transport mechanisms that will insure the continuous flow of iron from the environment into the microbes. The only species that do not require iron for life are the lactic acid-producing bacteria, which grow anaerobically and do not contain cytochrome (Macleod and Snell, 1947). Neilands (1972) has speculated that this exception represents a "regressive physiological adaptation" wherein these species evolved in an iron-poor environment and developed noniron means of forming deoxyribotides, namely vitamin B_{12} coenzyme.

2. Iron in nature

In the crust of the earth, iron stands fourth in abundance among all elements, ranking behind oxygen, silicon, and aluminum (Zajic, 1969). The iron composition in rocks is 3,800-56,300 ppm, in fresh waters 0.67 ppm, in sea waters 0.01 ppm, and in soils 38,000 ppm (Bowen, 1966).

Iron is found in two stable oxidation states, which are: ferrous iron with valence II and ferric iron with valence III (Spiro and Saltman, 1974). One property of iron that is of biological importance is its extreme insolubility, and the solubility varies between its ferrous and ferric forms. Ferric iron is 17 times more insoluble than ferrous iron due to its tendency to form polynuclear insoluble complexes (Spiro and Saltman, 1969). Iron in nature is usually present in the highly in-

soluble ferric form, and despite its abundance this insolubility makes iron utilization by living organisms difficult (Spiro and Saltman, 1969).

The insolubility of ferric iron and the importance of iron for microbial life demanded that microbes evolve special ferric ligands, which would dissolve, transport, and make iron available for biochemical functions of the microbes (Neilands, 1972). These ferric ligands are the siderophores.

3. Siderophores

Siderophore (Greek for "iron bearer"), a term first proposed by Lankford in 1973, is defined by Neilands (1981) as "a low-molecular-weight (500-1,000 daltons) virtually ferric-specific legand [sic], the biosynthesis of which is carefully regulated by iron and the function of which is to supply iron to the cell."

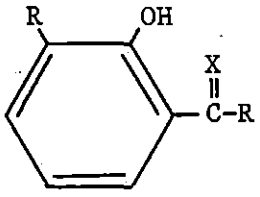
Siderophores have been found to be produced by aerobic or facultatively anaerobic bacteria and eukaryotic microorganisms (Neilands, 1981). Also, it has been found that some microbes produce more than one siderophore (Snow, 1970; Lankford, 1973). Only the lactic acid-producing bacteria, because they do not need iron, and the strict anaerobes, because iron in the low oxidation-reduction potential of the anaerobic environment is in the relatively soluble ferrous form, do not produce siderophores (Neilands, 1981).

The appearance of siderophores in cellular evolution is believed to be synchronous to the appearance of oxygen in the earth's atmosphere. Before that time, iron perhaps was in the more soluble ferrous form,

which was then oxidized to insoluble ferric form in the presence of atmospheric oxygen (Lankford, 1973).

The ability of the siderophores to bind ferric iron and transfer it to the microbe is due to their high affinity for the ferric iron, which ranges from $k_f = 10^{52}$ to $10^{22.9}$ (Neilands, 1981). Siderophores from different microbes have different chemical structures, although they all serve the same purpose (i.e., iron supply to cells), and this fact is attributed to the different evolutionary processes that took place in different microbes (Lankford, 1973).

The first siderophore to be isolated was obtained from Mycobacterium paratuberculosis (M. johnei) in 1949. Since then, numerous siderophores have been isolated and chemically characterized. Generally, the siderophores are classified as either the phenol-catechol type:



where X = H, OH and R = O, N

or the hydroxamic acid type: [-CO - $N(O^-)$-] (Neilands, 1981).

The phenol-catechol-type siderophores are divided into the cyclic catechols and linear catechols. Cyclic catechols include enterobactins isolated from Salmonella typhimurium (Pollack and Neilands, 1970) and Escherichia coli (under the name enterochelin) (O'Brien and Gibson, 1970). Linear catechols include agrobactin from the bacterial phytopathogen, Agrobacterium tumefaciens (Ong et al., 1979) and parabactin

from Micrococcus denitrificans (Tait, 1975).

The hydroxamic acid-type siderophores are divided into ferrichromes produced by the fungus Ustilago sphaenogena (Neilands, 1957) and the yeast Cryptococcus melibiosum (Atkin et al., 1970), rhodotorulic acids produced by yeasts (Atkin and Neilands, 1968; Atkin et al., 1970), citrate-hydroxamates produced by bacteria (Corbin and Bulen, 1969), mycobactins produced by mycobacteria (Francis et al., 1953; Snow, 1970), fusarinines produced by fungi (Emery, 1965), and ferrioxamines produced by streptomycetes (Bickel et al., 1960).

Besides the above-mentioned siderophores, a new kind of substance that may play a role in iron supply has been identified recently on the outer membrane of microbes. When grown under iron-poor conditions, several bacteria (E. coli, S. typhimurium, Shigella sp., Vibrio sp., Bordetella pertussis, and Neisseria gonorrhoeae) produce new outer membrane proteins (Griffiths, 1983; Payne, 1983; Simonson and DeVoe, 1983; Armstrong and Parker, 1984; West et al., 1984; Mietzner et al., 1984). The function of these new proteins has not been proven, but they may function as ferric iron-siderophore complex receptors (Payne, 1983), or as "novel nonsiderophore-mediated systems for acquisition of essential iron" (West et al., 1984).

4. Iron-storage compounds

The presence of iron-storage compounds in microbes was unknown until 1971, when the iron-binding protein, ferritin, was isolated from the fungus, Phycomyces blakesleeanus (David and Easterbrook, 1971). In

1979, ferritin also was isolated from Azotobacter vinelandii, expanding its occurrence to bacteria (Stiefel and Watt, 1979). Another iron-storage compound, hemin, was found in pigments of Yersinia pestis and was associated with virulence (Perry and Brubaker, 1984).

The level of ferritin in Phycomyces is regulated by the iron in the medium and increases 50-fold in iron-supplemented medium (David and Easterbrook, 1971). During spore germination, ferritin releases its iron, making it available for biosynthetic processes (David, 1974).

B. Iron-Binding Proteins in Mammals

1. Iron in mammals

Iron in mammals is found intracellularly and in body fluids (Finkelstein et al., 1983). The intracellular iron serves as a transporter of oxygen (hemoglobin in erythrocytes, myoglobin in muscles), as an activator of molecular nitrogen and oxygen (nitrogenases, oxygenases, oxidases), and as an electron transporter (cytochromes, iron-sulfur proteins) (Spiro and Saltman, 1974). Iron is also found in its storage form, bound to the protein ferritin, in such organs as liver, spleen, kidney, heart, muscle, bone marrow, placenta, and intestinal mucosa (Harrison et al., 1974). In body fluids, iron is bound to special proteins, such as transferrin in plasma (Morgan, 1974) and lactoferrin in mucosal secretions (Masson et al., 1966).

2. Iron-binding proteins

The above-mentioned proteins (hemoglobin, myoglobin, ferritin, transferrin, and lactoferrin) share the ability to bind iron, and so

they are called iron-binding proteins. Of these iron-binding proteins, transferrin and lactoferrin have been found to play a significant role in infection (Bullen et al., 1978), and more attention is given to them here.

Transferrin is a glycoprotein produced mainly by the liver of adult animals and by the lactating mammary glands of rabbits and rats (Morgan, 1974). Transferrin is included in the β-globulins of the plasma and normally circulates in plasma at 30% of its capacity to be saturated with ferric iron (Fe III) (Bullen et al., 1978). The main function of transferrin is transport of iron to the bone marrow for erythropoiesis and maintenance of iron balance in the body (Bullen et al., 1978). Transferrin binds free ionic iron from the plasma and acquires iron from body cells. Mechanisms by which iron is released from cells to transferrin are unknown. Perhaps enzymes or ascorbic acid facilitate iron release from the cells. Once released, iron is quickly bound to the free plasma transferrin (Morgan, 1974). The iron-transferrin complex delivers the iron to cells with the help of special receptors located on the cell surface. After binding the iron-transferrin complex, the receptor and the complex are internalized and reach an acidic vesicle inside the cell (Dautry-Varsat and Lodish, 1984). Because transferrin cannot bind iron in acidic environments below pH 4.5 (Surgenor et al., 1949), it releases its iron to the cell. The iron-free transferrin is returned to the cell surface and released by the receptor, which becomes available to bind another iron-transferrin complex (Dautry-Varsat and Lodish, 1984).

Lactoferrin is an iron-binding glycoprotein produced by mucosal surfaces, such as those in the nose, bronchi, and uterine cervix, and it also has been found in milk, saliva, tears, bronchial mucus, hepatic bile, pancreatic juice, seminal fluid, and urine (Masson et al., 1966). Lactoferrin also is a constituent of the specific granules of polymorphonuclear leukocytes (PMNs) (Masson et al., 1969; Pryzwansky et al., 1978). Like transferrin, lactoferrin binds two atoms of Fe III, but it will not release them in very acidic environments (Teuwissen et al., 1972).

Both transferrin and lactoferrin have been found to play a significant role in the defense mechanisms of mammals, because these proteins, if unsaturated, have a high affinity for iron (approximately 10^{30}) (Morgan, 1974), and they compete with microbial siderophores for available iron.

C. Role of Iron in Infections and Immunity

The first evidence that iron may play a role in infections was the observation of Schade and Caroline in 1944 that something in raw hen's egg inhibited the growth of Shigella dysenteriae and that this effect could be overcome only by the presence of iron. This inhibition was attributed to the presence of a protein, later called conalbumin (Bullen et al., 1974a), in the raw egg white. This protein was capable of binding with iron and making it unavailable to the microorganisms (Schade and Caroline, 1944). Two years later the same workers found the presence of another iron-binding protein in human plasma. Named transferrin, it

also inhibited the growth of S. dysenteriae (Schade and Caroline, 1946). These observations were the beginnings of the study of the role of iron in infections and immunity.

One of the necessities that microbes must satisfy when they enter their mammalian hosts is the need for iron. While iron is in the host's fluids in quantities greater than those needed by the microbes, almost all is bound to transferrin and lactoferrin. The free, dissociated, ionic iron is 10^8-fold less than that required for microbial growth (Weinberg, 1974). Therefore, the low levels of free iron in serum, due to the presence of transferrin, and in secretions, due to the presence of lactoferrin, have been associated with resistance to infections.

1. Iron levels and susceptibility to infections

It has been observed that starving humans or other animals suppress certain infections, like brucellosis and tuberculosis (Murray et al., 1976; Murray and Murray, 1977a). Also, differences in resistance to salmonellosis were observed in mice fed diets that differed in iron content. Puschmann and Ganzoni (1977) found that the diet low in iron produced lower levels of serum iron than did the normal diet, and that the rate of survival of mice infected with S. typhimurium was markedly higher in mice with low dietary iron. This increased survival was attributed to the unavailability of iron for microbial growth (Murray and Murray, 1977b).

In addition to the normal low level of free serum iron, which protects against microbial invasion to some degree, the host possesses other

mechanisms that further decrease serum iron available to microbes during infection. The first observation that serum iron is lowered during infection was in tuberculous patients, who were found to be hypoferremic (Locke et al., 1932). Numerous experiments have shown that hypoferremia occurs after infection (Weinberg, 1974; Bullen et al., 1978; Weinberg, 1978). For instance, experimental salmonellosis in calves and colibacillosis in chickens resulted in acute hypoferremia and an increase of the unbound iron-binding capacity of their blood (Piercy, 1979). The phenomenon of hypoferremia after infection is a part of the general concept of "nutritional immunity," which is defined as "the native and acquired abilities of an animal to restrict the availability of growth-essential nutrilites for use by parasitic microorganisms" (Kochan, 1977).

The mechanism by which hypoferremia occurs during an infection is not yet completely known. It has been found that during hypoferremia in rats a leukocytic endogenous mediator (LEM) released by PMNs causes a decrease in iron levels in the serum and an increase in iron levels in the liver (Pekarek et al., 1972). In another study, it was proposed that apolactoferrin (iron-free lactoferrin) released by PMNs removes iron from transferrin, and the resulting iron-lactoferrin complex is taken up by macrophages. The macrophages transport the iron for storage to RES (Van Snick et al., 1974). These two above mechanisms were not supported by another study, which found normal iron kinetics between transferrin-iron and erythrocyte heme-iron in the iron metabolic cycle of mice during the hypoferremia of meningococcal infection. This finding suggests that

hypoferremia may be due to "an impaired return of reticuloendothelial system-processed iron to the transferrin pool during the hypoferremic response" (Letendre and Holbein, 1983). Another finding relating to the mechanism of hypoferremia was the observed increased ferritin synthesis in the liver and spleen of rats during inflammation. This increase in levels of this iron-storage protein is believed to be the cause of the increasing retention of iron in RES storage during inflammation and to be a part of the general inflammatory process (Konijn and Hershko, 1977). Hypoferremia also accompanies pyrexia, which is another host defense response to infections. Febrile temperatures reduced the growth of bacteria in low-iron medium (Kluger and Rothenburg, 1979), and hypoferremia also followed the same circadian rhythm as pyrexia (Kluger et al., 1983). The return from hypoferremic to normal serum-iron levels is believed to be associated with the increased synthesis of transferrin and caeruloplasmin observed after the hypoferremic response. Caeruloplasmin facilitates the incorporation of iron into transferrin (Piercy, 1979; Beaumier et al., 1984).

To counteract the above-mentioned low level of iron available to microbes for their growth in mammalian hosts, infections still can occur by means of the production of microbial siderophores, which compete with transferrin for iron. The ability of microbes to produce siderophores is a virulence factor (Kochan, 1977; Bullen, 1983; Rogers, 1983) that can be illustrated well in the case of the avirulent strain of N. gonorrhoeae that becomes virulent in the presence of neisserial sidero-

phores from virulent strains (Finkelstein and Yancey, 1981).

In the converse situation, wherein iron is in excess and transferrin is saturated, there is a greater susceptibility to infections. Recrudescence of diseases like brucellosis and tuberculosis occurred in starving people after they began to eat. This phenomenon was attributed to the mobilization of stored iron and its increase in serum, causing saturation of the host's iron-binding capacity (Murray and Murray, 1977a; Murray and Murray, 1977b). Also, the increased incidence of infections in patients with thalassemia was related to the saturation of their serum iron-binding capacity, because their sera supported the growth of Candida albicans better than normal sera (Caroline et al., 1969). Perhaps a similar phenomenon may account for the high mortality due to pneumococcus in patients with sickle-cell anemia after a hemorrhagic crisis (Barrett-Connor, 1971). That excess of iron in the host increases the susceptibility to infections has been proved experimentally by the increasing severity of infections after neutralization of the host's hypoferremia with iron. Injection of iron into rabbits turns a nonlethal dose of Pseudomonas aeruginosa to a rapidly lethal infection (Bullen et al., 1974b), and injection of iron into guinea pigs has a similar effect on a nonlethal dose of E. coli (Bullen et al., 1968a). Lethal results also were obtained after iron injections into normal and vaccinated mice infected with Mycobacterium tuberculosis, in contrast to the low mortality seen in noniron treated mice (Kochan, 1974). Also, the number of Vibrio vulnificus required to produce lethal infections was smaller in iron-

treated mice (Simpson et al., 1984), and the lethal combination of hemoglobin and E. coli in intraperitoneal injection into mice (Bornside et al., 1968) was attributed to the iron excess supplied by the hemoglobin (Bullen et al., 1972).

Lactoferrin, the host's iron-binding protein found in many secretions, also is involved in "nutritional immunity" by making iron unavailable to microbes (Reiter and Oram, 1967). In milk, it has been shown that the degree of lactoferrin saturation with iron determines the amount of growth of Staphylococcus aureus. Ninety percent saturation did not prevent growth, while 12.5% did (Schade, 1974). In another study, resistance to infection in the bovine mammary gland after 150 hours of postlactational involution was related to the increased concentration of lactoferrin in the milk, while the high susceptibility of the gland prior to 90 hours of involution was related to the low concentration of lactoferrin in the milk (Smith and Schanbacher, 1977).

Protective capacity in vitro, similar to lactoferrin, was attributed also to ferritin, the iron-storage protein, when it was found that iron-free ferritin could inhibit the growth of virulent Neisseria meningitidis (Calver et al., 1979).

Interfering with the inhibitory role of transferrin and lactoferrin on the growth of microbes is a recently isolated, low-molecular weight, iron-binding compound named HAITF (host associated iron transfer factor). This factor, occurring in mammalian tissues, supported the growth of several pathogenic bacteria by supplying them with iron. The role of

HAITF in the pathogenesis of infections is not known yet (Jones et al., 1980).

2. Effects of iron on immunity

The involvement of iron, siderophores, and iron-binding proteins is not limited to the concept of "nutritional immunity." They also play a significant role in humoral and cell-mediated immunity of the host against microbial infections.

While it is generally accepted that iron promotes infections with its nutritional importance for microbial growth, it has been found that it can promote infections also by neutralizing the immune functions of the host. Injections of iron abolish the protective effect of antiserum against Clostridium welchii in guinea pigs (Bullen et al., 1967) and Pasteurella septica in mice (Bullen et al., 1968b). Whether there is an iron-neutralizable factor in immune animals is not known (Kochan, 1983), but it has been suggested that iron may neutralize acquired immunity by an action on the bacteriostatic system of transferrin, specific antibody, and complement against P. septica (Bullen et al., 1971), or transferrin and antibody against C. welchii (Bullen et al., 1967). Injections of iron also abolish the protective immunity of mice immunized against S. typhimurium (Kochan et al., 1978). This effect was attributed to the effect of iron on cellular immunity, because immune serum from these mice was not affected by the addition of iron. Iron was found to interefere with the expression, not the development, of acquired immunity, because iron had no effect if it was injected during the vaccination

period. It was suggested that this phenomenon implies "that iron does not interfere with the antigenic sensitization of T lymphocytes but intereferes with antibacterial activity of either lymphokines or macrophages" (Kochan, 1983). Recently, an iron-resistant immunity in mice against S. typhimurium was described by Kochan and Wagner (1984) who indicated that the serum from these mice possesses a factor, perhaps a lymphokine, that activates phagocytes to express iron-resistant activity.

Besides making iron unavailable to microbes, iron-binding proteins of the mammalian hosts play a role in the function of antibodies. The IgA immunoglobulin of bacteriostatic milk can act only in the presence of unsaturated lactoferrin (Rogers and Synge, 1978). Also, transferrin was found to be important for the action of IgG on E. coli in horses (Rogers, 1983). The mechanism by which transferrin and lactoferrin act in these cases is not clear. Because antibody against enterochelin, the siderophore of enteric bacteria, has been detected (Moore et al., 1980), it has been hypothesized that if an antibody directed against a microbial siderophore blocks the siderophoric iron-binding activity, the microbes may still acquire free iron via other mechanisms when the host's iron-binding proteins are saturated (Bullen et al., 1978). Finally, because both IgA immunoglobulin and lactoferrin occur on the surface of mucous membranes, they are considered to cooperate for protection of the intestinal and respiratory surfaces (Bullen, 1983).

Iron-binding proteins are also believed to play a role in the two functions of the lymphomyeloid system (surveillance and regulation) be-

cause of the presence of lactoferrin in neutrophils, ferritin in monocytes, and transferrin in lymphocytes (Sousa, 1983). For the protective role of lactoferrin, it has been found that lactoferrin from the specific granules of PMNs prevents neutralization of the bacteriocidal substances by iron (Gladstone, 1973). Also, lactoferrin enhances the hydroxyl radical production by human PMNs during phagocytosis (Ambruso and Johnston, 1981) and promotes aggregation of the PMNs and adherence to endothelial cells, retaining them at the inflammatory sites and therefore amplifying the inflammatory response (Oseas et al., 1981). On the other hand, introduction of iron into PMNs reverses their bacteriocidal action (Bullen and Wallis, 1977), which shows the importance of the unsaturated state of iron-binding proteins in providing the iron-free environment for the function of PMNs (Bullen et al., 1978).

D. Role of Iron in Parasitoses

1. Importance of iron for parasite's metabolism

Contrary to the large amount of information about the importance of iron for bacteria and fungi, knowledge about the requirements of parasites for iron is little and fragmentary. This fact is due to the greater difficulty of studying the more advanced metabolism and complex nutritional requirements of the parasites in comparison to microbes.

Bolla et al. (1972) observed that addition of hemoglobin, hematin, or hemin was required for the *in vitro* growth of free-living stages of *Nippostrongylus brasiliensis*, and they suggested that iron porphyrin

compounds are involved in the development of N. brasiliensis. Also, portions of the heme moiety of hemoglobin were found to be incorporated by in vitro-cultured Schistosoma mansoni schistosomules (Foster and Bogitsh, 1984), but the fate of the iron, as in the previous study, was not examined.

Entamoeba histolytica and Plasmodium falciparum are two parasites for which the importance of iron for their growth has been proven. E. histolytica in vitro has high iron requirements and can utilize inorganic iron in the form of $FeCl_3$ (Latour and Reeves, 1965) or organic iron in the form of ferric ammonium citrate, ferritin, or hemoglobin (Diamond et al., cited in Diamond et al., 1978). P. falciparum could not grow in vitro in the presence of desferioxamine (DF), which is a chelating agent specific for nonhemic iron. Therefore, DF was proposed as a possible treatment for malaria (Raventos-Suarez et al., 1982).

How parasites obtain the required iron is not known. There is no evidence that protozoa produce siderophores (Diamond et al., 1978), and no paper could be found reporting the production of siderophores in other groups of parasites. Diamond et al. (1978) have speculated that possible mechanisms of iron acquisition by the parasites may be the use of siderophores produced by microorganisms, the possession of a "low affinity nonspecific transport system," or the possession of mechanisms that can release iron from hemoglobin, ferritin, or transferrin.

2. Iron levels and susceptibility to parasitoses

Iron levels in the host are associated not only with susceptibility to bacterial and fungal infections but also to parasitoses. Murray et al. (1975a) first observed that children in eastern Niger with hyperferremia and high transferrin saturation (associated with their folate deficiency) commonly had attacks of cerebral malaria. Also, the same workers noticed that starving patients suffered attacks of P. falciparum malaria after their arrival in a hospital in eastern Niger. They suggested that this fact was due to hyperferremia and high transferrin saturation, which developed as a result of better nutrition in the hospital. Their suggestion is supported by their experimental results on rats, wherein rats injected with iron had a higher parasitemia with P. berghei (52%) and died at 14 days, whereas control rats had a lower parasitemia (38%) and died at 16 days (Murray et al., 1975b).

Similar differences in susceptibility were observed in amebiasis. African nomads made iron deficient because of their milk diet had less amebiasis than other nomads using mixed diets. When iron supplementation was given to the iron-deficient nomads, their susceptibility to amebiasis sharply increased. The milk that they consumed was found to have a low iron concentration and unsaturated lactoferrin and transferrin, which may compete with the amebae in the colon for any iron available (Murray et al., 1980). Also, hamsters overloaded with intraperitoneal or dietary iron had an increased incidence and severity of amebic hepatic lesions over controls (Diamond et al., 1978).

On the other hand, the host seems to react to parasitoses with the mechanism of "nutritional immunity" by trying to reduce iron availability to invading parasites. Tartour and Idris (1973) found that Zebu cattle infected with Trypanosoma congolense had an early hypoferremia and progressively reduced unsaturated iron-binding capacity (UIBC) and total iron-binding capacity (TIBC). Similar results, hypoferremia and decreased TIBC, were also observed in the sera of rats infected with T. lewisi (Lee et al., 1977a). However, deer mice infected with T. brucei did not have serum iron concentration different from their controls, but they had significantly increased iron sequestered in the spleen and liver (Victor and Kaneko, 1983). Decreased serum iron was also observed in splenectomized and nonsplenectomized donkeys infected with Babesia equi or B. caballi (Nafie et al., 1982). Furthermore, male humans with active hepatic amebic disease were found by Diamond et al. (1978) to have serum iron levels at 50% of those in healthy males. These workers, on the basis of their findings and the high iron requirements of E. histolytica, proposed that the reason the liver is the most common site of extra-intestinal amebiasis is the abundance of iron in the liver, which is the result of transfer of iron to the liver from the plasma. Also, the same workers proposed that the lower prevalence of hepatic amebic disease in females could be explained by their iron deficiency anemia due to menstruation and child bearing, both of which deplete iron stores. In addition, they proposed that the siderosis of the Zulus, due to the consumption of home-brewed beer prepared in iron cooking vessels, is the cause of the acuteness and fulminating nature of their amebic disease.

Another parasitism in which iron changes have been observed is fascioliasis. Sheep experimentally infected with Fasciola hepatica exhibited a gradual increase in TIBC, while the serum iron remained normal until a rapid fall began on post-infection day 147 (Sinclair, 1965). In another similar study of sheep experimentally infected with F. hepatica, the TIBC rose between 13 and 19 weeks, while the plasma iron was elevated during the first six weeks of exposure, but then decreased and was below normal (Gameel, 1982). The iron changes in the latter study were attributed to observed changes in plasma levels of ascorbic acid, which favors absorption of iron from the intestine, transportation of iron, and release of iron from storage sites. Plasma ascorbic acid levels decreased during the first 10 weeks of infection and then increased thereafter. Finally, in a study of serum iron levels in dogs infected with Ancylostoma caninum, it was not possible to determine to what extent the observed iron-deficiency anemia was due to host reaction and how much to blood-sucking by the hookworms (Vieira, 1974).

Contrary to the beneficial role of low iron in protecting the host against parasitoses, it has been found that dietary iron deficiency impairs the resistance of rats to T. lewisi by delaying the host's production of the reproduction-inhibiting antibody, ablastin (Lee et al., 1977b).

E. Role of Iron in Trichinellosis

Trichinellosis is the infection and resulting disease caused by the nematode parasite, Trichinella spiralis. Trichinellosis is a disease

affecting man and all mammals, domestic or wild (Campbell, 1983). The host becomes infected after ingestion of raw or undercooked meat containing the infective first-stage juveniles of T. spiralis. The infective juveniles are released from the meat in the host's stomach and 0.9 hours after digestion (Despommier, 1983) enter the cytoplasm of a row of columnar cells of the intestine (Wright, 1979). Ten to fourteen hours later, the juveniles molt to second-stage juveniles, at 15-22 hours they molt to third-stage juveniles, and at 23-30 hours they molt to fourth-stage juveniles, which become adults. It is not known if all moltings occur within the cytoplasm of the host's enterocytes (Despommier, 1983). The adults mate within the cytoplasm of a row of columnar enterocytes (Gardiner, 1976), and the females produce newborn juveniles. At this point, about 4-7 days after infection, the enteral phase of the life cycle of T. spiralis is completed, and the parenteral phase starts. The newborn first-stage juveniles migrate to the bloodstream via the lymphatics (Harley and Gallicchio, 1971), circulate and enter muscle cells, where they grow to about 1 mm in length without molting. The sarcoplasm around the growing juveniles becomes visibly altered, and such muscle cells have been called "nurse cells" (Despommier, 1983). After 15-30 days, the muscle juveniles become infective for the next host (Despommier, 1983).

Whether iron is important for the metabolic processes of T. spiralis in any of its life cycle stages it is not known. Also, it is not known if the host reacts to trichinellosis with "nutritional immunity" by try-

ing to reduce iron availability to any of the parasite's stages. The only report found dealt with an experimental study of the levels of trace elements in the spleen of rats infected with T. spiralis (Sismeev, 1975). It was found that the iron levels in spleens from trichinous rats were 3.5 times higher than those in uninfected control rats by day 60. This finding suggests that the host may react to trichinellosis with nutritional immunity.

III. OBJECTIVES

The purpose of this study was to investigate the role of iron in the pathogenesis of trichinellosis. To accomplish this, three experiments were undertaken, each with certain objectives.

Experiment I: To evaluate the importance of iron in the life of T. spiralis, the survival of T. spiralis muscle juveniles was measured in the presence or absence of free iron in their in vitro culture medium.

Experiment II: To detect whether the host reacts to trichinellosis with hypoferremia, pigs were inoculated with T. spiralis muscle juveniles, and their serum iron (SI) and total iron-binding capacity (TIBC) were measured periodically during the first 30 days of the infection. Also, total iron content of the livers and spleens were measured at necropsy.

Experiment III: To determine whether excessive iron in the host enhances the severity of trichinellosis, the number of T. spiralis juveniles in samples of muscles was compared between pigs injected with iron and pigs not given added iron. Also, histopathologic comparisons between these two groups of infected pigs were made.

IV. MATERIALS AND METHODS

A. Experiment I

In Experiment I, T. spiralis muscle juveniles were cultured axenically in vitro by using the method described by Meerovitch (1965). Ten culture flasks, each with approximately 500 juveniles, were used. In five of them, a strong chelator of iron, ethylenediamine-di(orthohydroxyphenyl acetic acid)(EDDA), which had been freed of extraneous iron, was added to the medium at a concentration of 1 mg/ml. The remaining flasks served as controls. The number of motile (live) and nonmotile (dead) juveniles in sample optical areas was measured daily under a dissecting microscope at 30 magnifications, and the survival rates for each flask were calculated. Which of the flasks contained EDDA was not known to the observer until each trial was complete in order to avoid bias. Evaluation was done until all juveniles were immotile (dead). The experiment was repeated two times, and statistical analysis was carried out by using the split-plot design. Also, in order to test if EDDA was toxic to juveniles (by means other than depriving them of iron), the experiment was repeated three times by including a group of flasks in which the medium contained enough iron to occupy the chelating effect of the EDDA. In the latter case, three flasks served as controls, three flasks contained EDDA, and three flasks contained iron-saturated EDDA.

1. Source of T. spiralis muscle juveniles

Washed live T. spiralis muscle juveniles were supplied by Dr. W. J. Zimmermann, Veterinary Medical Research Institute, Iowa State University.

Doses of 5,000 juveniles were inoculated into white rats *per os*. These rats served as the stock of *T. spiralis* muscle juveniles for subsequent experiments.

2. Preparation of *T. spiralis* muscle juveniles

T. spiralis muscle juveniles were digested free from ground muscle tissues collected from carcasses of infected white rats by means of an artificial gastric juice (7 ml HCl, 5 grams pepsin, 1000 ml H_2O, temperature 37°C) under constant mechanical agitation with plastic paddles for 48 hours. After the paddles were removed, the sediment was allowed to settle for one hour. Two-thirds of the supernatant fluid was siphoned off, and the remaining fluid and sediment were poured into a Baermann apparatus consisting of a 40-mesh screen fitted into the top of a 3-liter funnel. Water at 37°C was added to cover the screen. After one hour, the filtered mixture was washed several times by siphoning off the supernatant, adding water, and letting it sediment.

3. Sterilization of *T. spiralis* muscle juveniles

The apparatus used to sterilize the juveniles obtained from the muscle digests was constructed according to the method of Avery (1941), which employed migration through a bed of sand. A sand-filled chamber was made by closing one end of six-inch length of two-inch diameter glass tubing with a layer of cheesecloth held in place by a rubber band. A 3/8-inch layer of coarse sand (average particle size 0.7-1.8 mm) was added to the tube, and the whole was fitted to the stem of a one-liter funnel by means of rubber tubing, with a pinch clamp located between the funnel and the chamber. A 60-ml glass bottle fitted by a stopper below the sand chamber

served as the collector receptable for juveniles that had migrated through the sand chamber. All components of the apparatus excluding the funnel were autoclaved before each use. Ringer's saline solution (0.85% aqueous solution of NaCl) with 0.5% $NaHCO_3$ (added to neutralize acid filtering through the sand) was sterilized by filtration through a 0.22 micron filter without grid (Falcon) under vacuum and added to the collection bottle, which was attached below the sand-filled chamber.

The artificial gastric juice containing the T. spiralis juveniles was poured into the funnel, and the pinch clamp was opened gradually so that the flow of fluid into the sand-filled chamber would not disturb the sand. The entire apparatus was placed in an incubator at 37°C for four to five hours, allowing the juveniles to migrate into the Ringer's saline solution in the bottle. After closing the pinch clamp, the collection bottle was separated from the sand-filled chamber, and the juveniles were washed in sterilized Tyrode's solution. The sterility of the juveniles obtained from the filtration was tested by incubation of 1 ml of the solution on blood agar plates at 37°C for 12 hours.

4. Preparation of the basal medium

The basal medium was the same as used by Meerovitch (1965) and consisted of one part of heat-inactivated normal rabbit serum (GIBCO Laboratories), and four parts of 25% chick-embryo extract (CEE_{25}). The rabbit serum was inactivated by heating in a water bath at 56°C for 30 minutes. The CEE_{25} was prepared before each use by diluting CEE_{50} (GIBCO Laboratories) 1:1 with sterilized Tyrode's solution (pH 7.2). The Tyrode's

solution was prepared by diluting 9.6 grams of Tyrode's salts (Sigma Chemical Co.) in 900 ml of deionized distilled water. One gram of $NaHCO_3$ was added, and the pH of the solution was adjusted to 7.0 by using 1 N HCl or 1 N NaOH while stirring. Addition of deionized distilled water brought the volume to 1000 ml. The solution then was sterilized by filtering it through a 0.22 micron filter without grid (Falcon) under vacuum. The final pH was 7.2. The Tyrode's solution was tested for sterility by incubating one ml on blood agar plates at 37°C for 12 hours. The final medium had a pH value of approximately 7.2. Penicillin sulfate (Eli Lilly and Co.) and streptomycin G potassium (Squibb) were added in addition to what was already present in the CEE_{50}, so that the final concentration was approximately 100 i.u./ml for the former and 100 µg/ml for the latter.

5. Preparation of EDDA free of iron

EDDA (Sigma Chemical Co.) was freed from contaminating iron by the method of Rogers (1973). Ten grams of EDDA were dissolved in 190 ml of boiling 1 N HCl. After cooling, the solution was filtered through filter paper and diluted with 1500 ml of acetone. The pH of the acetone solution was raised to 6.0 by adding 1 N NaOH. After standing overnight at 4°C, the precipitate was collected by filtration through paper, washed with cold acetone, and allowed to air dry.

6. Incubation of cultures

Cultures of T. spiralis juveniles were made in sterile screw-capped tissue-culture flasks having a 25-cm^2 growth area (Falcon). Each flask contained 2.5 ml of medium, into which 0.2 ml of Tyrode's solution con-

taining approximately 500 T. spiralis muscle juveniles was inoculated. In addition, the medium in half of the flasks contained 1 mg/ml EDDA. The flasks were incubated in a shaking water bath (GCA/Precision Scientific, Model 50) at 37°C and 20 oscillations per minute. Upon termination of cultures, the medium was checked for its pH and tested for sterility by incubation on blood agar plates at 37°C for 12 hours.

B. Experiments II and III

Experiments II and III were conducted simultaneously by using eight barrow and one gilt feeder pigs weighing about 18-22 kg each. The pigs were divided randomly into three groups of three pigs each, housed separately in concrete-floored pens, and given water and commercial feed ad lib. Each pig in two groups was inoculated per os with 100,000 T. spiralis muscle juveniles, and the remaining group served as the control. The source of T. spiralis muscle juveniles was the same as described in Experiment I. One group of pigs inoculated with T. spiralis muscle juveniles also was injected with 0.5 ml of iron dextran (Ferrextran ^{R}, Fort Dodge Laboratories, Inc.) subcutaneously (SC) at intervals of three to four days. Injection sites (inguinal and axillary regions) were rotated over the course of the trial. The remaining two groups were injected with 0.5 ml of Ringer's solution placebo.

About 5 ml of blood was taken from the anterior vena cava of each pig three times per week for 30 days. The blood samples were centrifuged for five minutes within one hour after their collection, and the serums were transferred to clean labelled tubes and stored at -5°C. To facili-

tate separation of serum from the coagulate, glass beads were added to the blood samples before their centrifugation.

After the experiment was completed, the serum samples were assessed for serum iron (SI) and total iron-binding capacity (TIBC) by using an autoanalyzer.[1] The unsaturated iron-binding capacity (UIBC) was calculated by using the formula UIBC = TIBC - SI, and the saturation concentration (SC) was calculated by using the formula SC = SI/TIBC x 100. The pigs were euthanized by electrocution on post-infection days 34, 38, and 40, and the weight of each pig was recorded. At necropsy, whole livers and spleens were removed and assessed for total iron content by using the dry ash atomic absorption method (Stahr, 1977). Also, samples were collected from the following muscles: diaphragm, tongue, masseter, deltoideus, pars thoracica mi. trapezii, brachialis, extensor carpi radialis, epaxial muscle, obliquus externus abdominalis, psoas, gluteus medius, glutebiceps, semimembranosus, semitendinosus, heart, and smooth muscles from stomach. After grinding, 50 g of each muscle sample were digested as described in Experiment I, and juvenile counts for each sample were made. In addition, small samples collected from the above-mentioned muscles were fixed in 10% neutral-buffered formalin, sectioned at 5 μ and stained with hematoxylin and eosin for histopathologic examinations.

Serum iron levels, total iron-binding capacities, unsaturated iron-

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[1] Technicon AutoAnalyzer II™. Method No. 25a, May 1974. Technicon Instruments Corporation/Tarrytown, NY 10591.

binding capacities, and saturation concentrations were statistically compared among the three groups of pigs by using the split-plot design. The total iron content and iron concentration in the livers and spleens were compared among the three groups of pigs by using the randomized complete block design, the muscle juvenile counts by using the t-test, and the weight gains by using the one-way analysis. Also, histopathologic evaluations of skeletal, cardiac, and smooth muscles were statistically compared among the infected pigs by using the nested with repeated measurements design on the measurements of length, width, and number of nuclei in eight randomly selected "nurse cells" from each pig.

V. RESULTS

A. Experiment I

Experiment I, the objective of which was to determine whether iron is important for the survival of T. spiralis muscle juveniles, was executed in two phases. In the first phase, the survival rates of the juveniles were compared in control or EDDA-containing culture mediums (replications 1 and 2), and in the second phase, the survival rates of the juveniles were compared in control and EDDA-, or EDDA plus Fe-containing culture mediums (replications 3, 4, and 5).

Results for replications 1 and 2 are presented in Table 1 and Figure 1, and results for replications 3, 4, and 5 are presented in Table 2 and Figure 2.

As can be seen from Figures 1 and 2, the mean survival rates of the control groups were much higher than the survival rates of the groups cultured in the EDDA-containing mediums, with one exception (replication 5). Statistical analysis showed that this effect of EDDA on juvenile survival rates is highly significant ($P = 0.0016$ for replication 1, $P = 0.0001$ for replications 2 and 3, and $P = 0.0005$ for replication 4). In replication 5, no difference was found between control and EDDA-containing mediums, probably due to the fact that, by the time this replication was performed, the T. spiralis muscle juveniles used had been stored in a refrigerator for one month, and therefore their viability was low.

Table 1. Survival rates (%) of T. spiralis muscle juveniles cultured in vitro in EDDA

Hours		20		44		68	
Medium		Control	EDDA	Control	EDDA	Control	EDDA
Replication 1							
No. of flask	1	62.42	28.26	44.23	0.00	21.53	7.27
	2	72.22	73.14	34.16	0.73	32.11	0.97
	3	48.10	36.48	31.14	0.00	12.50	0.00
	4	60.57	34.37	33.33	0.00	29.20	0.00
	5	65.68	53.08	61.34	0.00	60.00	1.30
Mean		62.99	45.06	40.84	0.14	31.06	1.90
Replication 2							
No. of flask	1	78.67	13.69	87.40	2.77	77.44	0.00
	2	80.00	8.16	62.08	0.63	69.39	2.20
	3	74.64	49.68	54.06	0.00	61.45	0.00
	4	66.25	33.74	75.70	0.00	63.75	0.69
	5	73.72	61.13	62.18	4.58	68.04	0.00
Mean		74.65	33.82	68.28	1.59	68.01	0.57

92		116		140		164	
Control	EDDA	Control	EDDA	Control	EDDA	Control	EDDA
12.28	0.00	7.01	3.60	6.66	0.99	0.00	0.88
13.33	0.66	6.66	0.00	3.47	0.00	0.00	0.00
5.74	0.00	0.00	0.00	4.16	0.00	1.73	0.00
12.61	0.00	0.00	0.00	3.57	0.00	0.00	0.00
27.20	1.81	15.25	0.00	3.80	2.02	3.54	0.00
14.23	0.49	5.78	0.72	4.33	0.60	1.05	0.17
60.69	0.42	48.97	0.00	27.63	0.00	3.80	0.00
79.08	0.00	65.67	0.00	15.60	0.00	2.96	0.00
49.35	0.00	41.73	0.00	9.35	0.00	1.70	0.00
57.46	0.40	52.29	0.00	7.63	0.00	1.58	0.00
75.53	0.00	36.97	0.93	25.74	0.00	0.63	0.00
64.42	0.16	49.12	0.18	17.19	0.00	2.13	0.00

Figure 1. Effect of EDDA on the survival of T. spiralis muscle juveniles in vitro

a) Replication 1
b) Replication 2

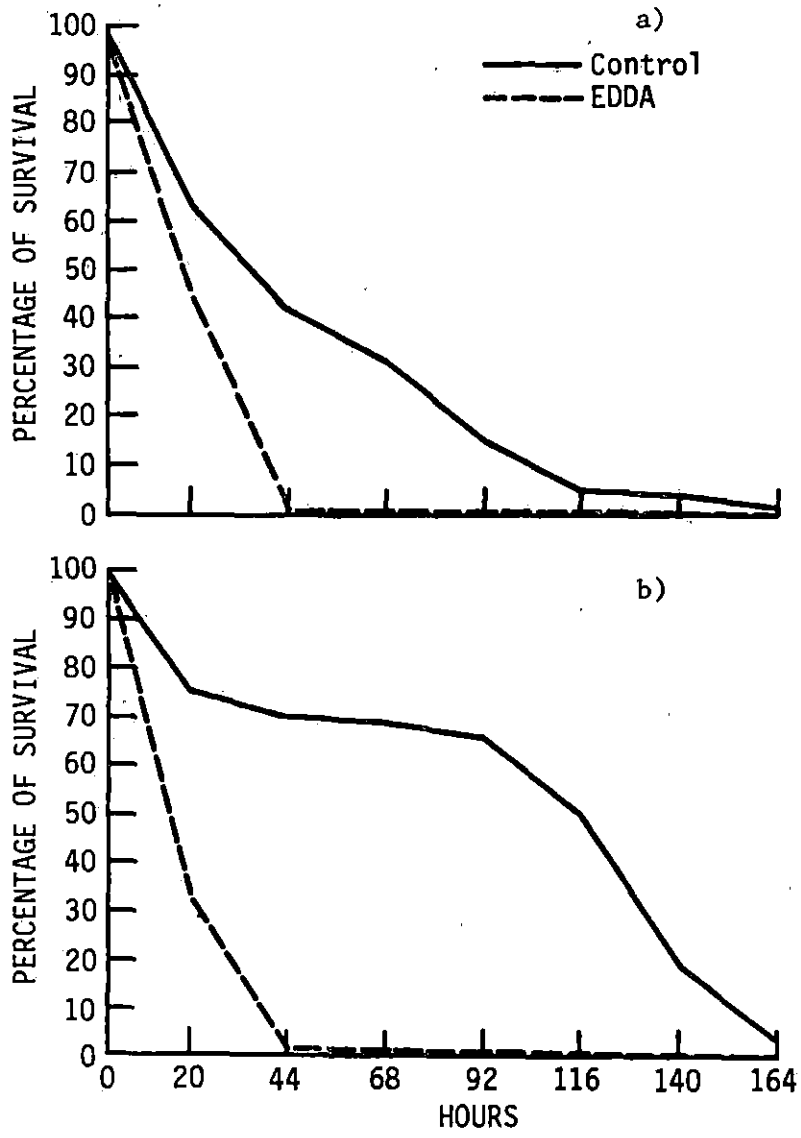

a)
Control
EDDA
PERCENTAGE OF SURVIVAL
100
90
80
70
60
50
40
30
20
10
0
b)
PERCENTAGE OF SURVIVAL
0
20
44
68
92
116
140
164
HOURS

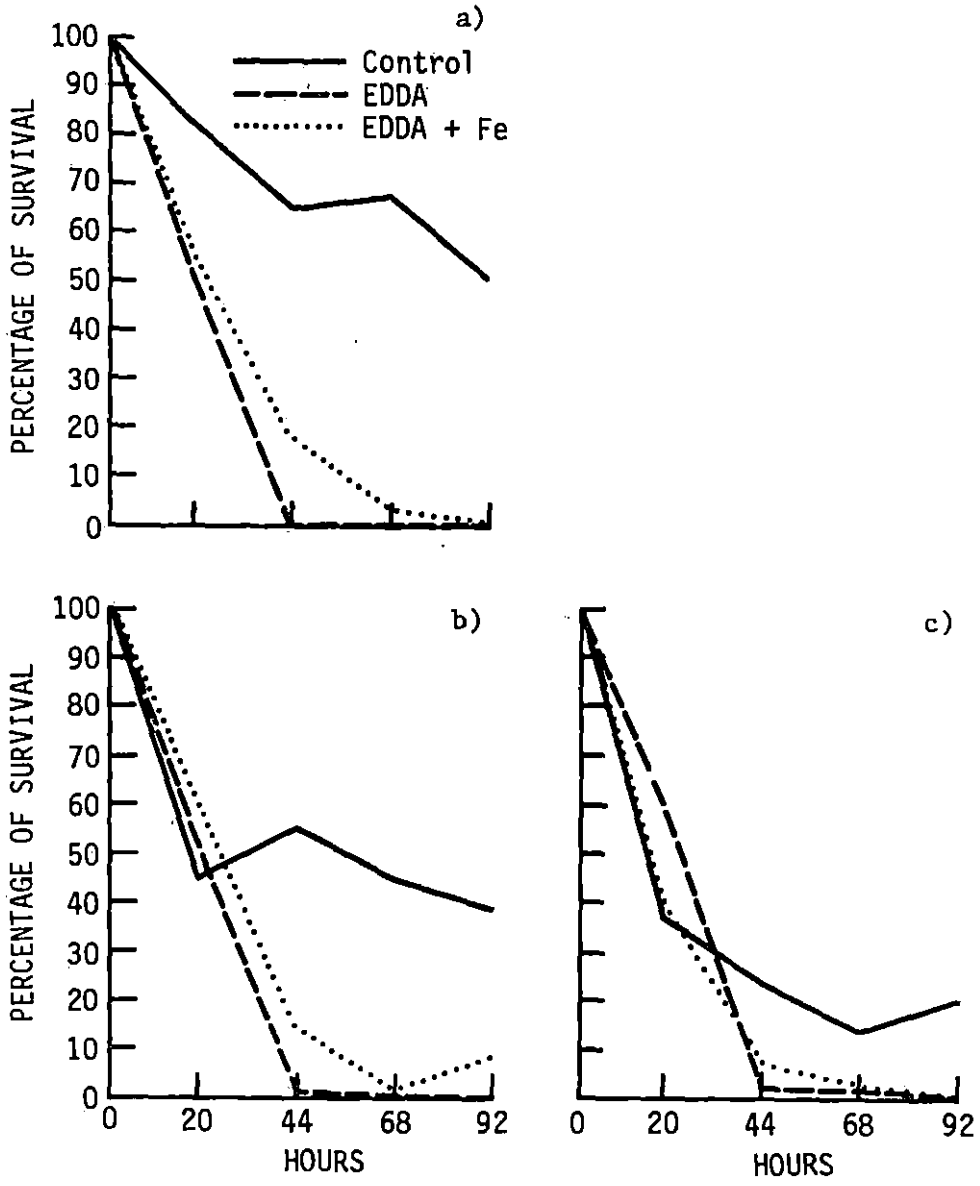
Table 2. Survival rates (%) of T. spiralis muscle juveniles cultured in vitro in EDDA or EDDA plus Fe

Hours		20			44		
Medium		Control	EDDA	EDDA+Fe	Control	EDDA	EDDA+Fe
Replication 3							
No. of flask	1	79.71	46.19	42.30	64.96	0.00	11.32
	2	85.91	32.31	57.14	59.31	0.00	11.84
	3	80.53	69.93	51.06	70.92	0.00	26.47
Mean		82.05	49.47	50.16	65.05	0.00	16.54
Replication 4							
No. of flask	1	22.58	38.77	56.75	57.57	2.65	12.00
	2	33.61	54.44	65.68	48.64	0.00	6.79
	3	79.23	67.27	59.03	61.31	0.78	24.46
Mean		45.40	53.49	60.48	55.84	1.14	14.41
Replication 5							
No. of flask	1	40.00	78.22	43.28	8.95	0.00	10.58
	2	25.56	33.57	29.37	29.72	1.51	7.07
	3	46.45	67.66	31.48	29.28	5.12	5.88
Mean		37.33	59.81	37.71	22.65	2.21	7.84

68			92		
Control	EDDA	EDDA+Fe	Control	EDDA	EDDA+Fe
64.86	0.00	3.33	46.90	0.00	0.00
71.42	0.00	2.98	48.35	0.00	0.00
67.64	0.00	0.00	58.75	0.00	0.00
67.97	0.00	2.10	51.33	0.00	0.00
52.67	0.00	2.43	35.91	0.00	11.01
39.34	0.00	0.00	36.45	0.00	5.40
42.27	0.00	0.00	44.44	0.00	12.94
44.76	0.00	0.81	38.93	0.00	9.78
0.00	1.28	6.34	12.76	0.00	0.00
0.00	0.00	0.00	0.00	0.00	0.00
38.52	2.46	1.16	47.95	0.00	0.00
12.84	1.24	2.50	20.23	0.00	0.00

Figure 2. Effect of EDDA or EDDA plus Fe on the survival of *T. spiralis* muscle juveniles *in vitro*

a) Replication 3
b) Replication 4
c) Replication 5


a)
Control
EDDA
EDDA + Fe
PERCENTAGE OF SURVIVAL
100
90
80
70
60
50
40
30
20
10
0
b)
c)
0
20
44
68
92
HOURS

On the other hand, in comparing the effect of EDDA and EDDA plus Fe on juvenile survival, no significant differences were found. It must be mentioned, however, that the pH in the latter group of flasks was not maintained at 7. Although the pH was balanced at 7 at the start of the experiment, it always fell to 5 by the end of the experiment. Attempts to prevent this, namely the use of different iron compounds like $FeCl_3$ and $FeNH_4(SO_4)_2$, were ineffective. The cause of this pH alteration is not explained.

At the end of each replication, samples of T. spiralis were drawn from the control, EDDA- and EDDA plus iron-containing mediums and examined under a microscope. Molting of the juveniles was observed in the control and the EDDA plus iron-containing mediums, but the juveniles had died before molting could occur in the EDDA-containing medium. Evidence of molting was recognized by detecting a separation of the sheath from the cuticle, usually at the anterior and posterior ends of the worm (Figure 3).

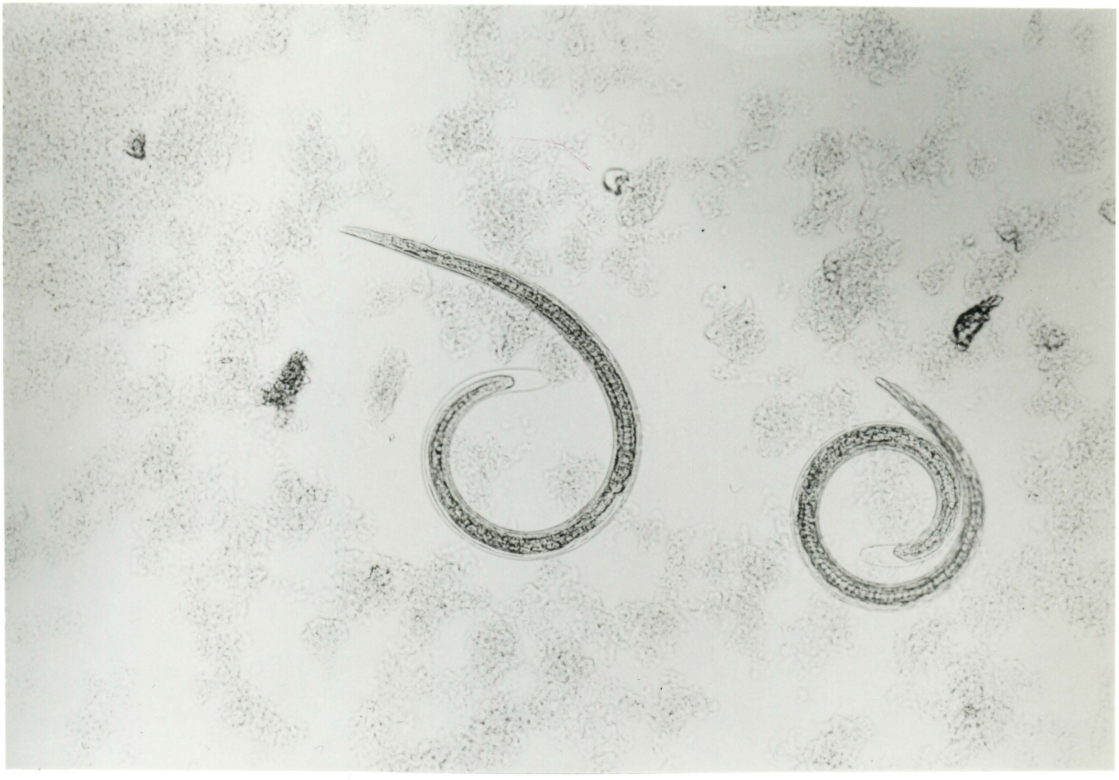
B. Experiment II

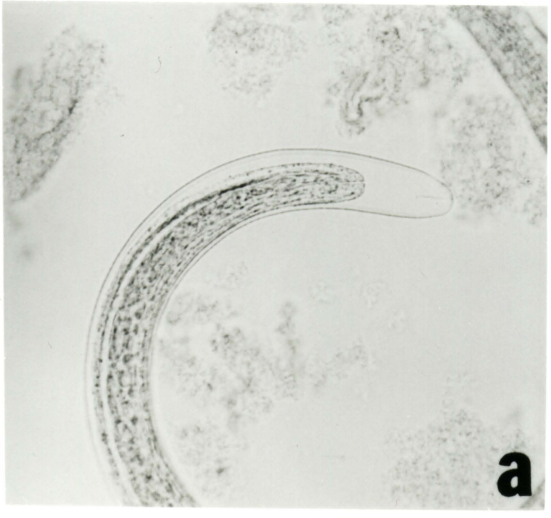
In Experiment II, the objective was to evaluate whether the host reacts to trichinellosis with hypoferremia.

Serum iron (SI), total iron-binding capacity (TIBC), unsaturated iron-binding capacity (UIBC), and saturation concentration (SC) were measured in trichinous (Group A), control (Group B), and iron-injected trichinous pigs (Group C) on days -2, 0, 1, 5, 7, 10, 17, 24, and 40 post infection.

Figure 3. Molting of T. spiralis juveniles in vitro in EDDA plus iron-containing medium after 92 hours of incubation (120X)

a. Anterior end (216X)
b. Posterior end (216X)




a

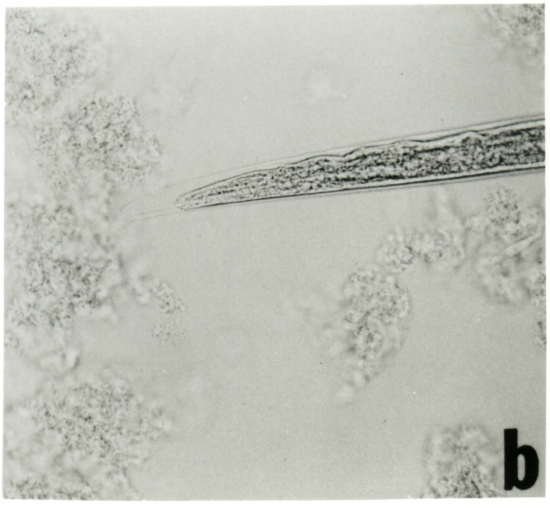

b

Table 3 presents the above-mentioned values for the three groups of pigs over the course of the experiment. Figures 4, 5, and 6 show the SI and SC curves, and Figures 7, 8, and 9 show the UIBC and TIBC curves for groups A, B, and C, respectively.

Statistical analysis showed that trichinellosis or trichinellosis and iron injections had no significant effect on the levels of SI and SC of the three groups of pigs. On the other hand, TIBC levels in the control groups were significantly higher than those in the trichinous pigs ($P = 0.015$) and in the iron-injected trichinous pigs ($P = 0.0432$), but there was no difference between the infected groups. Also, UIBC levels in the control pigs were significantly higher than those in the trichinous pigs ($P = 0.0152$) and in the iron-injected trichinous pigs ($P = 0.0568$), but there was no difference between the two infected groups. The above-mentioned differences in TIBC and UIBC levels between control and infected groups occurred during the first 17 days post infection.

Table 4 presents iron concentration and total iron content in the livers and spleens of experimental pigs at necropsy.

Statistical analysis showed that the levels of iron concentration in the livers and spleens were significantly different among the three groups ($P = 0.003$). Liver iron concentration levels were highest in the iron-injected group and lowest in the trichinous group. The iron concentration levels in the spleens were highest in the iron-injected group and lowest in the control group.

On the other hand, there was no significant difference among the

Table 3. Serum iron (SI), total iron-binding capacity (TIBC), unsaturated iron-binding capacity (UIBC), and saturation concentration (SC) of serum samples from experimental pigs

Group	No. of pig		Days -2	Days 0
Trichinous pigs	1	SI[a]	206	170
		TIBC[a]	382	695
		UIBC[a]	176	525
		SC[b]	53.92	24.46
	2	SI	151	75
		TIBC	357	592
		UIBC	206	517
		SC	42.29	12.66
	3	SI	183	98
		TIBC	379	659
		UIBC	196	561
		SC	42.28	14.82
Control pigs	4	SI	206	154
		TIBC	623	869
		UIBC	417	715
		SC	33.06	17.72
	5	SI	226	94
		TIBC	604	667
		UIBC	378	573
		SC	37.41	14.09
	6	SI	144	86
		TIBC	549	695
		UIBC	405	609
		SC	26.22	12.37

[a] Expressed in µg Fe/100 ml serum.

[b] Expressed in percentage.

[c] * = sample not enough for SI or TIBC measurement.

[d] - = could not be calculated because of missing data for SI or TIBC.

Days						
1	5	7	10	17	24	40
142	78	37	100	124	89	65
*[c]	762	629	576	535	467	469
-[d]	684	592	476	411	378	404
-	10.23	5.88	17.36	23.17	19.05	13.85
101	90	28	41	42	63	39
530	539	571	497	494	472	546
429	449	543	456	452	409	507
19.05	16.69	4.90	8.24	8.50	13.34	7.14
60	123	146	48	169	147	171
467	472	*	481	357	535	621
407	349	-	433	188	388	450
12.84	26.05	-	9.97	47.33	24.42	27.53
168	143	101	218	247	250	203
661	908	1001	782	818	815	613
493	765	900	564	571	565	410
25.41	15.74	10.08	27.87	30.19	30.67	33.11
*	133	79	146	124	185	156
502	756	700	667	609	664	569
-	623	621	521	485	479	413
-	17.59	11.28	21.88	20.36	27.86	27.41
133	156	87	92	119	147	125
530	705	508	511	563	500	705
397	549	421	419	444	353	580
25.09	22.12	17.12	18.00	21.13	29.40	17.73

Table 3. continued

Group	No. of pig		Days -2	Days 0
Fe-injected trichinous pigs	7	SI	114	57
		TIBC	343	628
		UIBC	229	571
		SC	33.23	9.07
	8	SI	205	26
		TIBC	486	733
		UIBC	281	707
		SC	42.18	3.54
	9	SI	178	95
		TIBC	489	859
		UIBC	764	390
		SC	36.40	11.05

Days						
1	5	7	10	17	24	40
37	68	51	73	96	103	108
423	541	363	398	426	494	567
386	473	312	325	330	391	459
8.74	12.56	14.04	18.34	22.53	20.85	19.04
28	62	46	28	60	75	90
645	664	456	426	461	439	446
617	602	410	398	401	364	356
4.34	9.33	10.08	6.57	13.01	17.08	20.17
91	137	100	113	107	101	183
481	577	541	481	297	409	613
390	440	441	368	190	308	430
18.91	23.74	18.48	23.49	36.02	24.69	29.85

Figure 4. Changes in serum iron (SI) and saturation concentration (SC) in trichinous pigs

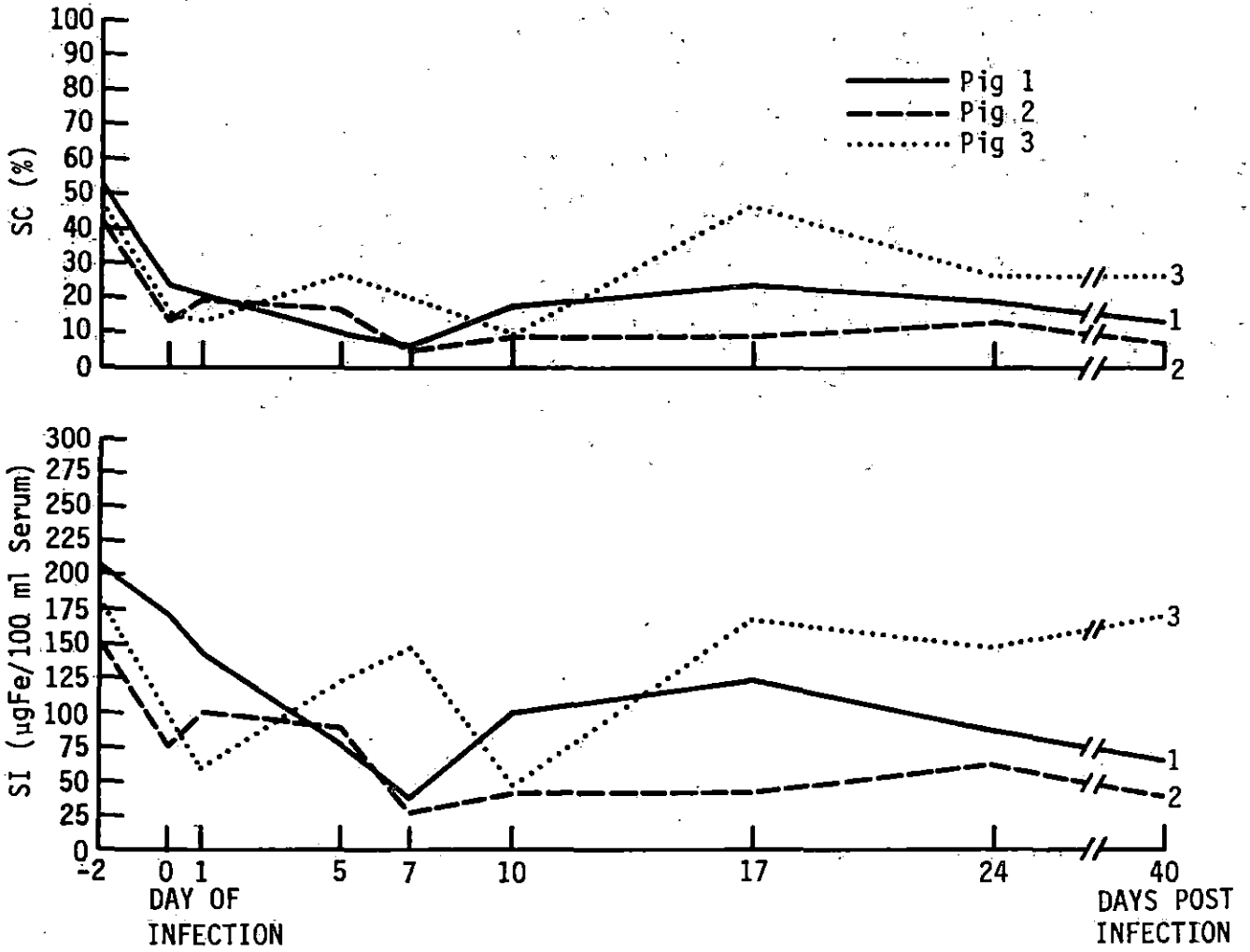

Pig 1
Pig 2
Pig 3
SC (%)
100
90
80
70
60
50
40
30
20
10
0
3
1
2
SI (µgFe/100 ml Serum)
300
275
250
225
200
175
150
125
100
75
50
25
0
-2
0
1
5
7
10
17
24
40
DAY OF INFECTION
DAYS POST INFECTION

Figure 5. Changes in serum iron (SI) and saturation concentration (SC) in control pigs

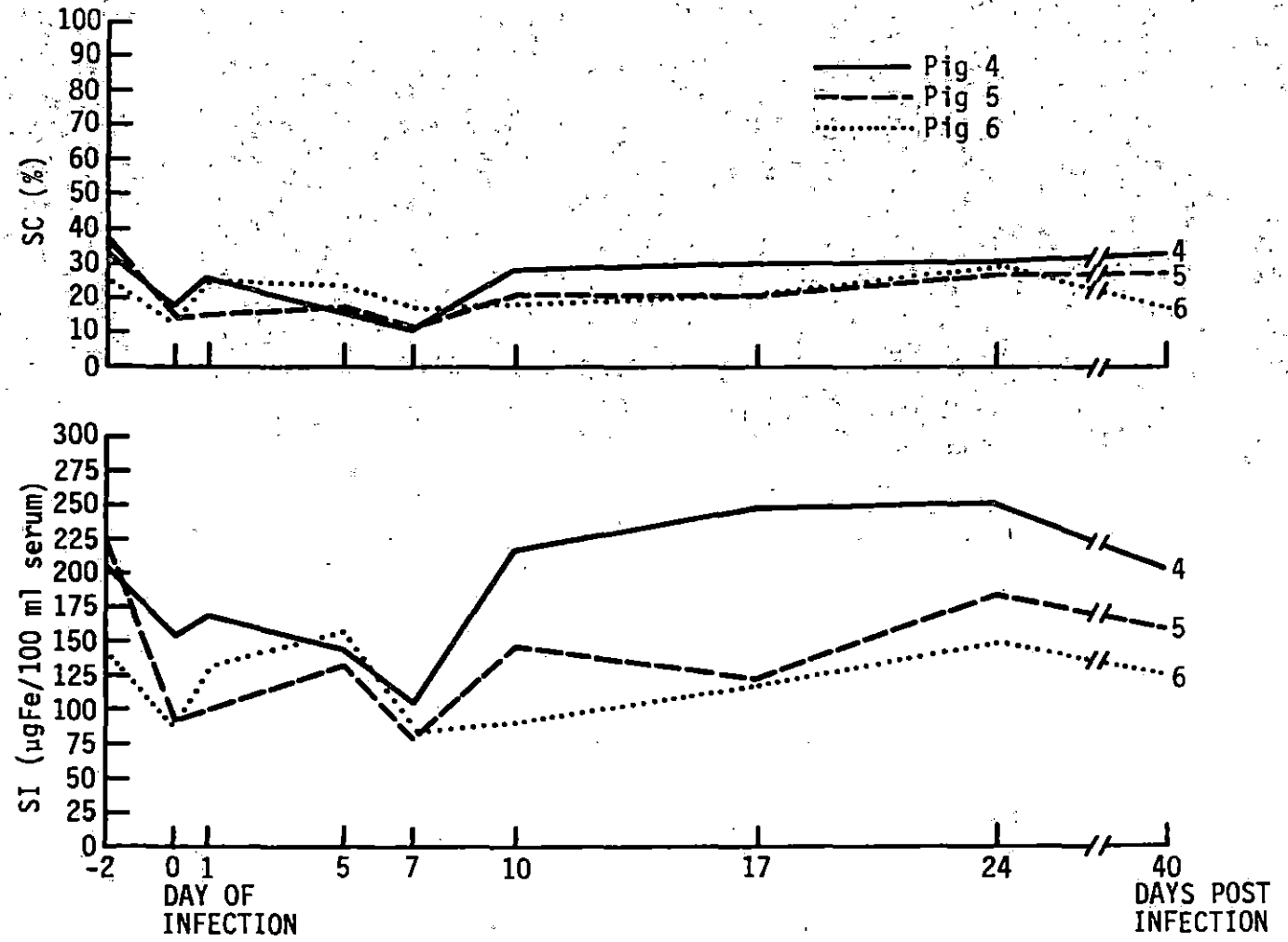

Pig 4
Pig 5
Pig 6
SC (%)
SI (µgFe/100 ml serum)
4
5
6
-2
0
1
5
7
10
17
24
40
DAY OF INFECTION
DAYS POST INFECTION

Figure 6. Changes in serum iron (SI) and saturation concentration (SC) in Fe-injected trichinous pigs

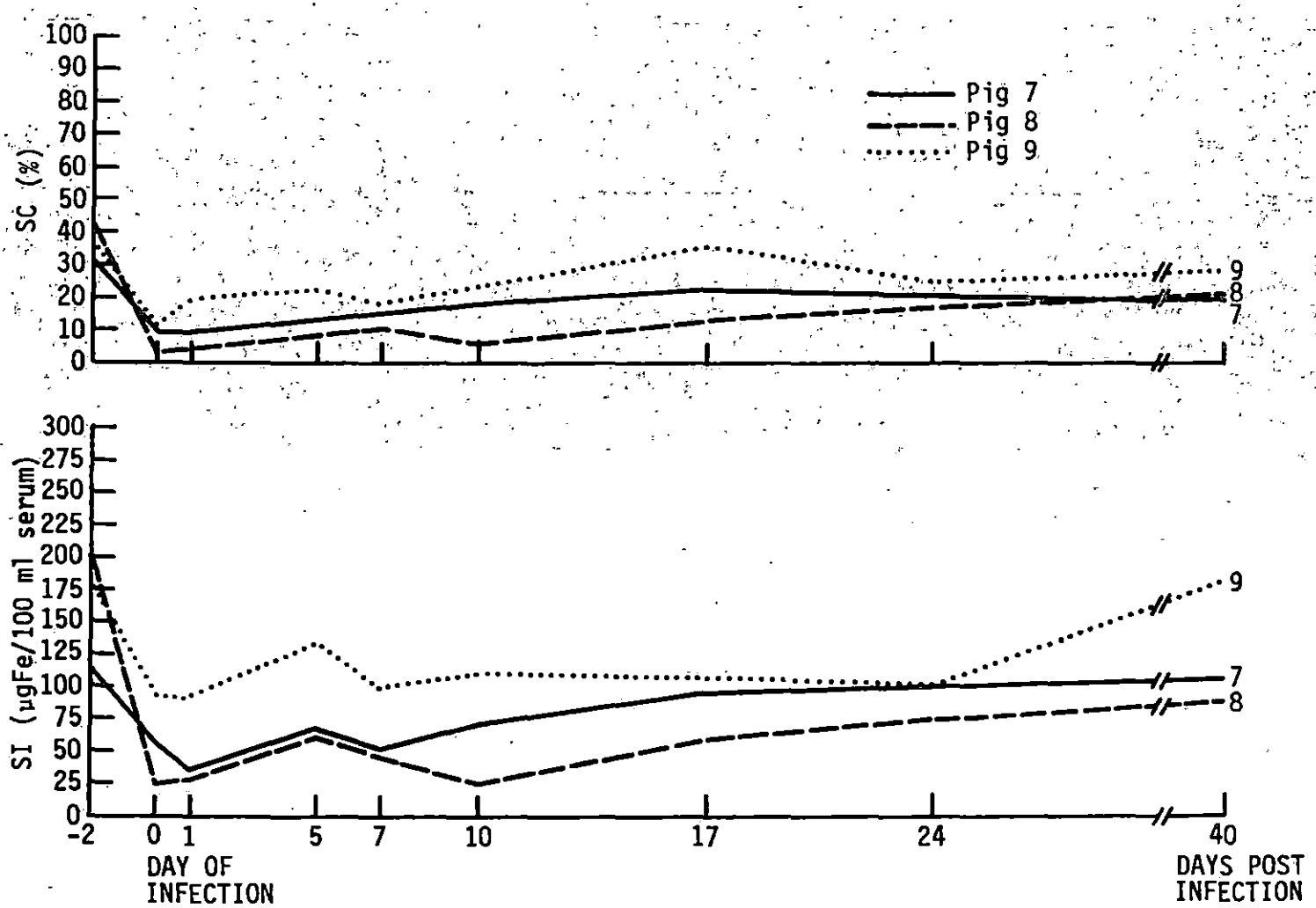

Pig 7
Pig 8
Pig 9
SC (%)
100
90
80
70
60
50
40
30
20
10
0
9
8
7
SI (µgFe/100 ml serum)
300
275
250
225
200
175
150
125
100
75
50
25
0
9
7
8
-2
0
1
5
7
10
17
24
40
DAY OF INFECTION
DAYS POST INFECTION

Figure 7. Changes in unsaturated iron-binding capacity (UIBC) and total iron-binding capacity (TIBC) in trichinous pigs

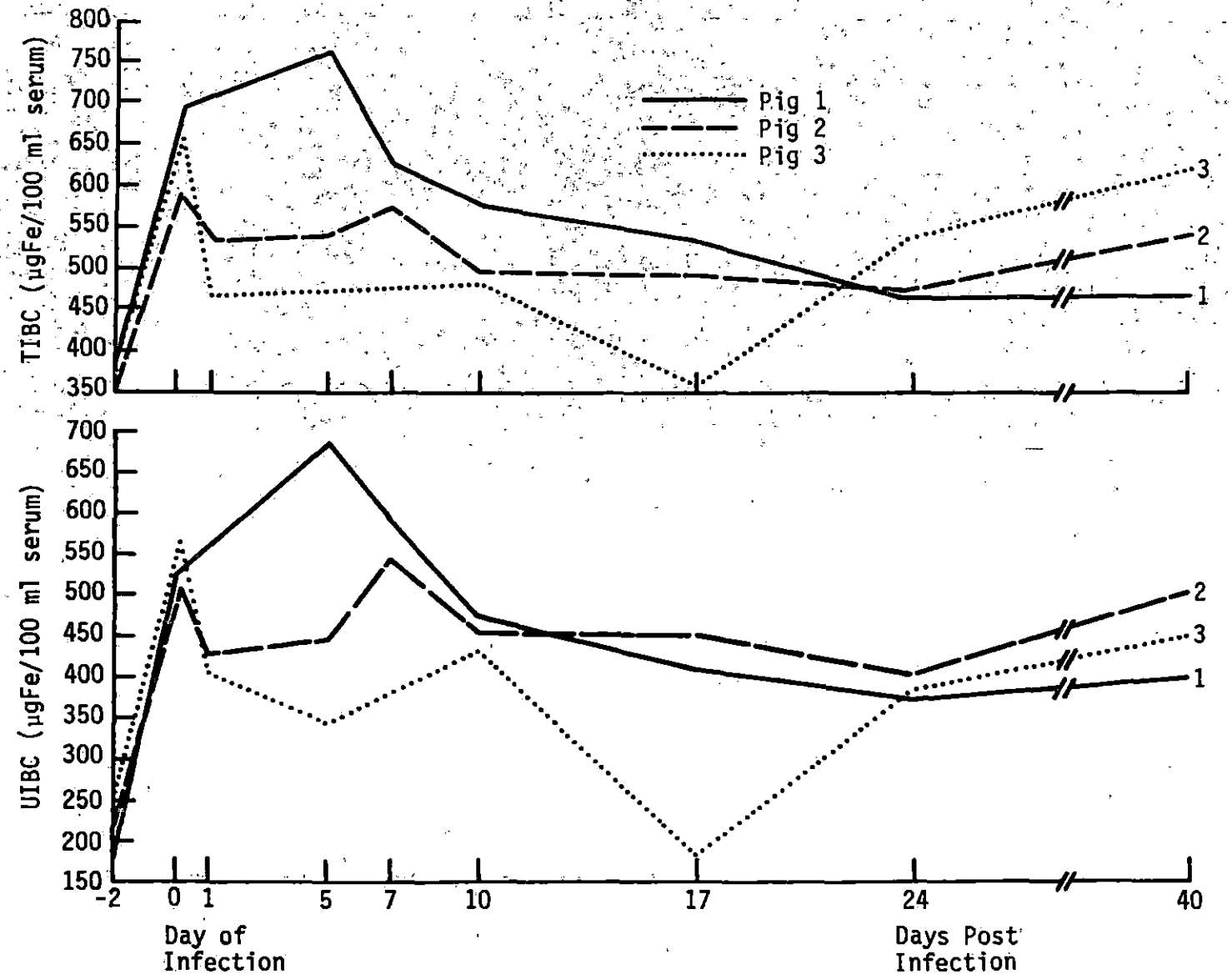

TIBC (µgFe/100 ml serum)
UIBC (µgFe/100 ml serum)
Pig 1
Pig 2
Pig 3
800
750
700
650
600
550
500
450
400
350
300
250
200
150
-2
0
1
5
7
10
17
24
40
Day of Infection
Days Post Infection

Figure 8. Changes in unsaturated iron-binding capacity (UIBC) and total iron-binding capacity (TIBC) in control pigs

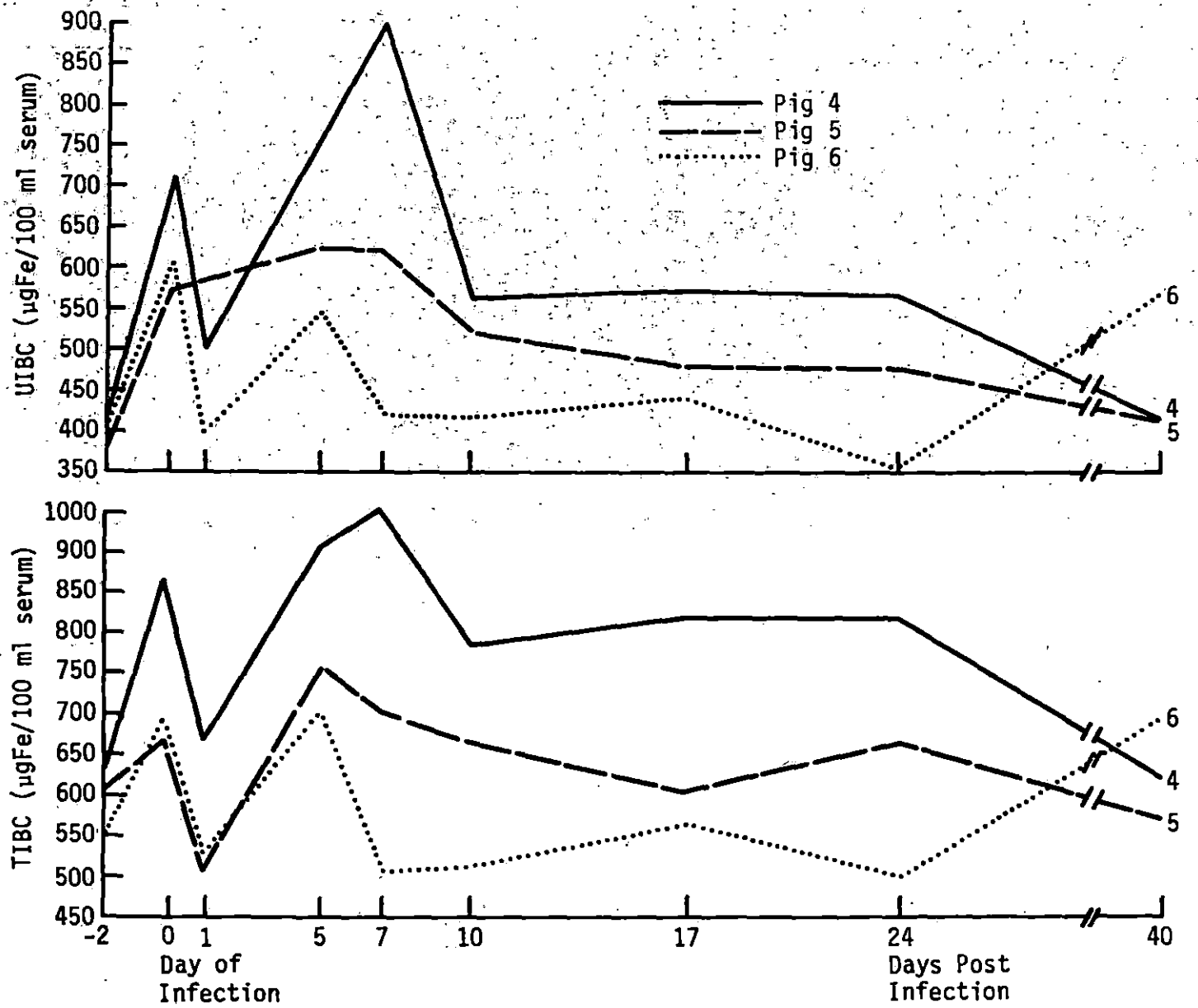

UIBC (µgFe/100 ml serum)
900
850
800
750
700
650
600
550
500
450
400
350
Pig 4
Pig 5
Pig 6
6
4
5
TIBC (µgFe/100 ml serum)
1000
900
850
800
750
700
650
600
550
500
450
6
4
5
-2
0
1
5
7
10
17
24
40
Day of Infection
Days Post Infection

Figure 9. Changes in unsaturated iron-binding capacity (UIBC) and total iron-binding capacity (TIBC) in Fe-injected trichinous pigs.

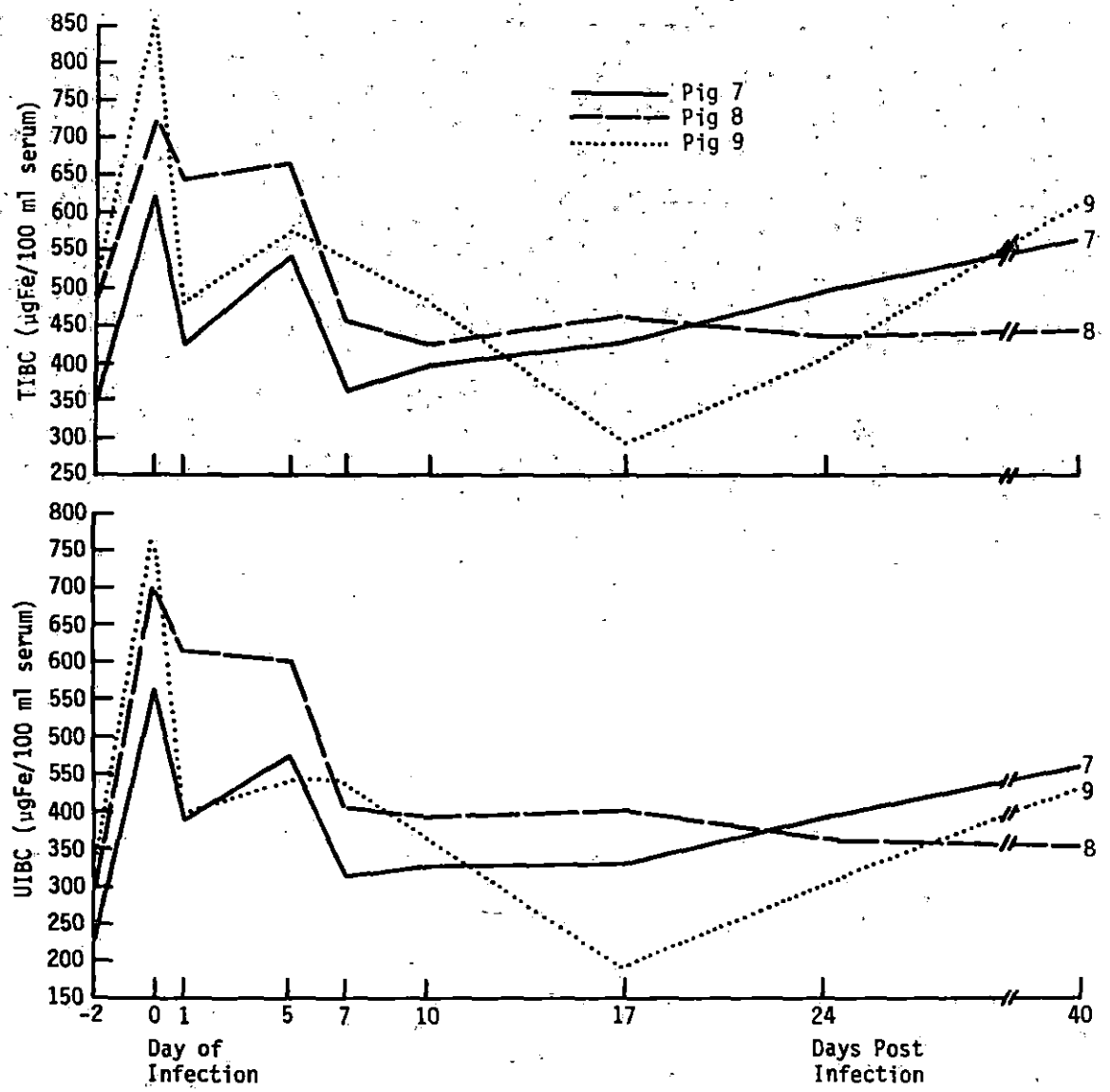

Pig 7
Pig 8
Pig 9
TIBC (µgFe/100 ml serum)
UIBC (µgFe/100 ml serum)
850
800
750
700
650
600
550
500
450
400
350
300
250
200
150
9
7
8
-2
0
1
5
7
10
17
24
40
Day of Infection
Days Post Infection

Table 4. Concentration of Fe in livers and spleens, as well as weight and total iron for spleens of experimental pigs

Group	No. of pig	Liver Fe (ppm)	Spleen Fe (ppm)	Spleen Weight (g)	Spleen Total Fe (ppmxg)
Trichinous pigs	1	84	130	72.63	9441.90
	2	58	130	55.92	7269.60
	3	160	78	52.42	4088.76
	Mean	100.66	112.66		6933.42
Control pigs	4	100	62	52.77	3271.74
	5	130	68	60.26	4097.68
	6	140	76	47.92	3641.92
	Mean	123.33	68.66		3670.44
Fe-injected trichinous pigs	7	160	160	62.18	9948.80
	8	180	150	60.08	9012.00
	9	200	130	39.05	5076.50
	Mean	180.00	146.66		8012.43

three groups of experimental pigs regarding total iron content of the spleens. The total iron content of the livers could not be calculated, because their weights were mistakenly not recorded.

C. Experiment III

In Experiment III, the objective was to evaluate whether injection of iron in the host affects the severity of trichinellosis, the distribution of T. spiralis muscle juveniles, the dimensions and number of nuclei in "nurse cells," and the weight gain of each experimental pig.

Table 5 presents the number of T. spiralis muscle juveniles per gram of tissues collected at necropsy of pigs, each of which had been given 100,000 juveniles. No juveniles were found in the control group. Table 6 presents the length, width, and number of nuclei in eight randomly selected "nurse cells" from these tissues. Table 7 presents the weight gain of each experimental pig during the course of the experiment.

Statistical analysis showed that there were no differences between the two infected groups of pigs in the number of juveniles per gram of muscle or in the dimensions and nuclei number of "nurse cells." Also, the weight gains among the three groups of experimental pigs were not statistically different.

Table 5. Distribution of T. spiralis muscle juveniles per gram of tissues from infected pigs

Group	No. of pig	Muscles			
		Diaphragm	Tongue	Masseter	Shoulder[a]
Trichinous pigs	1	2860	2930	1857.14	1300
	2	1660	910	530.30	500
	3	1240	1240	909.09	310
Fe-injected trichinous pigs	7	610	550	250	240
	8	7060	3479.59	2428.57	2350
	9	4600	4620	1775	1760.86

[a]Includes: Pars thoracica mi. trapezii and m. deltoideus.

[b]Includes: m. obliquus internus abdominis and m. obliquus externus abdominis.

[c]Includes: m. semimembranosus and m. semitendinosus.

Muscles						Mean	
Dorsal epaxial m.	Belly[b]	Psoas	Hind leg[c]	Heart	Stomach	Each pig	Group
550	1700	3280	1140	0.00	0.00	1561.71	
430	470	660	400	0.00	10.00	557.03	906.21
420	520	580	470	30.00	0.00	559.90	
290	200	280	220	0.00	0.00	264.00	
1860	3930	3220	1860	0.00	0.00	2618.81	1558.79
1270	1410	1410	1090	0.00	0.00	1793.58	

Table 6. Dimensions (μ) and number of nuclei in eight randomly selected "nurse cells" in tissues from infected pigs

Group	No. of pig		No. of sample "nurse cell" 1st	2nd	3rd
Trichinous pigs	1	Length	490	500	350
		Width	150	140	120
		No. of nuclei	16	24	13
	2	Length	430	500	590
		Width	160	150	140
		No. of nuclei	7	15	20
	3	Length	500	440	1500
		Width	160	170	180
		No. of nuclei	11	18	17
Fe-injected trichinous pigs	7	Length	450	450	400
		Width	140	140	140
		No. of nuclei	13	13	12
	8	Length	800	550	350
		Width	150	140	150
		No. of nuclei	14	10	15
	9	Length	550	500	400
		Width	150	150	180
		No. of nuclei	21	24	19

No. of sample "nurse cell"					Mean	
4th	5th	6th	7th	8th	Each pig	Group
400	470	600	650	750	526.25	
130	140	130	140	150	137.50	
21	10	12	20	20	17	
750	950	950	450	400	627.50	594.58
150	120	140	120	120	137.50	146.25
20	18	22	3	8	14.12	15.24
600	550	350	540	560	630.00	
140	170	190	110	190	163.75	
15	23	8	17	8	14.62	
550	340	530	690	500	488.75	
160	150	150	140	160	147.50	
8	14	19	14	12	13.12	
350	500	850	900	700	625.00	530.00
160	160	180	160	140	155.00	155.41
9	15	9	15	24	13.87	13.33
460	450	200	750	500	476.25	
120	160	250	150	150	163.75	
7	5	9	11	8	13.00	

Table 7. Weight gain (%) of experimental pigs

Group	No. of pig	Weight (kg)		Gain (%)	
		Start	Finish	Each pig	Group mean
Trichinous pigs	1	17.55	33.30	89.74	
	2	20.70	27.90	34.78	65.67
	3	18.00	31.05	72.50	
Control pigs	4	20.70	39.15	89.13	
	5	19.80	32.85	65.90	69.06
	6	20.70	31.50	52.17	
Fe-injected trichinous pigs	7	22.05	40.05	81.63	
	8	22.50	22.95	2.00	37.40
	9	18.90	24.30	28.57	

VI. DISCUSSION AND CONCLUSIONS

A. Experiment I

In the present study, it was observed that the presence of EDDA reduces drastically the survival rates of the T. spiralis muscle juveniles in vitro. This could be attributed to the ability of EDDA to bind iron, which is known to be important for aerobic metabolism, making it unavailable to them. On the other hand, addition of iron in the EDDA medium did not increase the survival, perhaps due to the development of very low pH of 5 during the experiment.

The possible importance of iron for the survival of the muscle juveniles in vitro indicates that it may be involved in oxygen consumption. This implication is in contrast to the observations of Ferguson and Castro (1973) that T. spiralis muscle juveniles survive anaerobically in vitro for several days, while adults cannot live anaerobically beyond 12 hours. Specifically, they observed that T. spiralis adults consume about 14 µl O_2/mg protein per hour, while the oxygen uptake of the juveniles was negligible. Also, Meerovitch (1965) found that development of juveniles in vitro was better in the presence of CO_2 than O_2. The apparent contradiction of the present study with the above-mentioned studies can be answered by the explanation given by Stewart (1983), who reported that muscle juveniles exposed to digestive fluid for times exceeding 45 minutes may have changed their metabolism from that of juveniles to that of adults. In the Ferguson and Castro (1973) study, the muscle juveniles remained in the digestive fluid for 45 minutes, while

in the present study they remained for 18 hours. In the Meerovitch (1965) study, the muscle juveniles remained in the digestive fluid for three to four hours, and at 48 hours incubation under air they had a mean survival of 38%, while in the present study at 44 hours it was 50.53%. It seems, therefore, that the juveniles in the present study, after remaining for so long in the digestive fluid, had advanced more to the adult-type aerobic metabolic pattern, and this can explain the possible importance of iron for them. This explanation is supported by the finding that juveniles in the control and EDDA plus Fe culture mediums exhibited molting.

Another observation that derives from the present study concerns the speculation of Diamond et al. (1978) that the mechanism of iron acquisition by parasites may be through the use of siderophores produced by microorganisms
siderophores). This hypothesis was not supported by the results in these experiments with T. spiralis, because juveniles in the control cultures had a long survival without the presence of demonstrable microorganisms, and therefore T. spiralis can utilize iron without the help of microorganisms.

In conclusion, it seems that iron may be important for the survival of only those stages of T. spiralis that possess an adult-type aerobic metabolic pattern.

B. Experiment II

In the present study, reductions in total iron-binding capacity (TIBC) and unsaturated iron-binding capacity (UIBC), coupled with no difference in the serum iron (SI) and saturation concentration (SC) levels of the infected pigs, were observed in comparison to the control group. The cause of these reductions was not determined.

Decreased TIBC levels have been observed in rats infected with Trypanosoma lewisi (Lee et al., 1977a) and in Zebu cattle infected with T. congolense (Tartour and Idris, 1973). In the latter case, decreased UIBC was also observed. The cause of these reductions is not clear, but it has been proposed that the half-life of transferrin may be shortened; the liver may be unable to meet the demand for transferrin; or the synthesis, storage, and/or mobilization of transferrin may be affected so that the replenishment of the protein is impaired (Tartour and Idris, 1973). These explanations perhaps may also apply in the present study.

Regardless of the cause of TIBC and UIBC reductions, it seems that these reductions are associated with the molting of the T. spiralis juveniles, the mating of the adults, and the early migration of the newborn juveniles, because the changes occurred in the first 17 days post infection. This association may be correlated with the observation in Experiment I that iron may be important for the survival of T. spiralis juveniles that have advanced toward the adult-type aerobic metabolic pattern. It is not known if there is any causal relationship between importance of iron for T. spiralis and inability of the host to maintain

constant TIBC and UIBC levels.

Also, in this study, it was found that the iron concentration in the spleens of trichinous pigs is higher than that in control pigs. This is similar to the observation of Sismeev (1975), who reported that spleens of trichinous rats have higher iron levels than spleens of uninfected control rats. On the other hand, it was observed that there were no differences among the total iron content of spleens in the three groups of experimental pigs, due to the unequal weights of the spleens. The last observation suggests that the notion that the host may react to trichinellosis with nutritional immunity because of the spleen's increased iron concentration has doubtful validity.

Another observation was that the livers of the trichinous pigs had the lowest iron concentration, while the livers of the iron-injected trichinous pigs had the highest. The high iron concentration in the iron-injected trichinous pigs may be linked to their overload with iron, while no explanation is apparent explaining the very low iron concentration in the livers of the trichinous pigs.

In conclusion, it seems that pigs do not react to trichinellosis with hypoferremia.

C. Experiment III

In the present study, it was found that iron injections in trichinous pigs had no effect on the number of T. spiralis muscle juveniles per gram of tissues or on the dimensions and number of nuclei in "nurse cells" in comparison to placebo injections. The distribution of the

juveniles in both infected groups of experimental pigs agrees with that described by Zimmermann and Schwarte (1961) and Olsen et al. (1964). Possible explanations for the above-mentioned observations are: a) the iron could not reach the T. spiralis worms, b) the iron does not affect the reproductive potential of T. spiralis adults, and/or c) the iron does not affect the development of the "nurse cells."

Even though the mean numbers of T. spiralis muscle juveniles per gram of tissues in the two infected groups of experimental pigs are not statistically different, a closer look at Table 5 reveals that pigs 2 and 3 in the trichinous group had a low mean number of muscle juveniles per gram of tissues, while pig 1 had a relatively high number. Also, pigs 8 and 9 had a high mean number of muscle juveniles per gram of tissues, while pig 7 had a relatively low number. Pigs 2, 3, 8, and 9 support the hypothesis that the addition of iron may increase the severity of trichinellosis, while pigs 1 and 7 behave exactly the opposite. It is not known if this observation has any validity or not, because the number of experimental pigs per group was small, and we do not know if pigs 1 and 7 were an anomaly or not.

Finally, no difference in the weight gains was found among the three groups of experimental pigs. This observation is in contrast to the observation of Stewart (1976) who reported that trichinous mice had lower weight gains over uninfected control mice. No explanation is apparent for this contradiction.

In conclusion, it seems that the addition of iron to swine tissue does not affect the severity of their infection with trichinellosis.

VII. SUMMARY

In the present study, the role of iron in the pathogenesis of trichinellosis was examined.

First, it was found that the survival of T. spiralis muscle juveniles is markedly shortened in the presence of the strong iron chelator, EDDA. This finding suggests that iron may be important for the metabolism of T. spiralis.

Second, it was found that, after infection of pigs with trichinellosis, their total iron-binding capacity (TIBC) and unsaturated iron-binding capacity (UIBC) are reduced during the first 17 days of infection, in comparison to uninfected control pigs. This was true whether or not the pigs had received iron injections during the course of infection.

Third, the splenic iron concentration in Trichinella-infected pigs was increased over the noninfected controls, but not the total iron content.

The last two observations suggest that pigs cannot react to trichinellosis with hypoferremia.

Finally, no difference was found in the per gram number of muscle juveniles or in the structure of "nurse cells" between trichinous and iron-injected trichinous pigs. Also, no difference was found in the weight gains between trichinous, iron-injected trichinous, and uninfected control pigs. These observations suggest that the addition of iron in trichinous pigs does not affect the severity of infection.

VIII. LITERATURE CITED

Ambruso, D. R.; Johnston, R. B. Lactoferrin enhances hydroxyl radical production by human neutrophils, neutrophil particulate fractions, and an enzymatic generating system. J. Clin. Invest. 67:352-360; 1981.

Armstrong, S. K.; Parker, C. D. Effects of iron starvation on Bordetella pertussis. Abstracts of the Annual Meeting-1984: Abstract D18. American Society for Microbiology. 84th Annual Meeting, St. Louis, Missouri, 4-9 Mar. 1984.

Atkin, C. L.; Neilands, J. B. Rhodotorulic acid, a diketopiperazine dihydroxamic acid with growth-factor activity. I. Isolation and Characterization. Biochemistry 7:3734-3739; 1968.

Atkin, C. L.; Neilands, J. B.; Phaff, H. J. Rhodotorulic acid from species of Leucosporidium, Rhodosporidium, Rhodotorula, Sporiodiobolus, and Sponbolomyces, and a new alanine-containing ferrichrome from Cryptococcus melibiosum. J. Bacteriology 103:722-733; 1970.

Avery, L. A simple method of removing bacteria that adhere to trichina larvae. Proceedings of the Helminthological Society 8:6-7; 1941.

Barrett-Connor, E. Bacterial infection and sickle cell anemia. Medicine 50:97-112; 1971.

Beaumier, D. L.; Caldwell, M. A.; Helbein, B. E. Synthesis of transferrin (Tf) and Caeruloplasmin (Cp) during experimentally induced hypoferremia in mice. Abstracts of the Annual Meetings-1984: Abstract B118. American Society for Microbiology. 84th Annual Meeting, St. Louis, Missouri, 4-9 Mar. 1984.

Bickel, Von H.; Gäumann, E.; Keller-Schirlein, W.; Prelog, V.; Vischov, E. Über eisenhaltige wachstumsfaktoren und ihre antagonisten. Experientia 16:128-168; 1960.

Bolla, R. I.; Weinstein, P. P.; Lou, C. In vitro nutritional requirements of Nippostrongylus braziliensis. I. Effects of Sterols, Sterol Derivatives and Heme Compounds on the Free Living Stages. Comp. Biochem. Physiol. 43B:487-501; 1972.

Bornside, G. H.; Buis, P. T. T.; Cohn, I. J. Hemoglobin and Escherichia coli, a lethal intraperitoneal combination. J. Bacteriology 95:1567-1571; 1968.

Bowen, H. J. M. Trace elements in Biochemistry. New York: Academic Press; 1966.

Bullen, J. J. Significance of iron in infection. Schlessinger, D., ed. Microbiology-1983. Washington, D.C.: American Society for Microbiology; 1983; 327-328.

Bullen, J. J.; Wallis, S. N. Reversal of the Bacterial effect of polymorphs by a ferritin-antibody complex. FEMS Letters 1:117-120; 1977.

Bullen, J. J.; Cushnie, G. H.; Rogers, H. J. The abolition of the protective effect of Clostridium welchii type A antiserum by ferric iron. Immunology 12:303-312; 1967.

Bullen, J. J.; Leigh, L. C.; Rogers, H. J. The effect of iron compounds on the virulence of Escherichia coli for guinea pigs. Immunology 15:581-588; 1968a.

Bullen, J. J.; Wilson, A. B.; Cushnie, G. H.; Rogers, H. J. The abolition of the protective effect of Pasteurella septica antiserum by iron compounds. Immunology 14:889; 1968b.

Bullen, J. J.; Rogers, H. J.; Lewin, J. E. The bacteriostatic effect of serum on Pasteurella septica and its abolition by iron compounds. Immunology 20:391-406; 1971.

Bullen, J. J.; Rogers, H. J.; Griffiths, E. Iron binding proteins and infection. Brit. J. Haem. 23:389-392; 1972.

Bullen, J. J.; Rogers, H. J.; Griffiths, E. Bacterial iron metabolism in infection and immunity. Neilands, J. R., ed. Microbial iron metabolism. New York: Academic Press; 1974a; 517-551.

Bullen, J. J.; Ward, C. G.; Wallis, S. N. Virulence and the role of iron in Pseudomonas aeruginosa infection. Infection and Immunity 10:443-450; 1974b.

Bullen, J. J.; Rogers, H. J.; Griffiths, E. Role of iron in Bacterial infection. Current Topics in Microb. Immun. 80:1-35; 1978.

Calver, G. A.; Kenny, C. P.; Kushner, D. J. Inhibition of the growth of Neisseria meningitidis by reduced ferritin and other iron-binding agents. Infection and Immunity 25:880-890; 1979.

Campbell, W. C. Epidemiology. I. Modes of Transmission. Campbell, W. C., ed. Trichinella and Trichinellosis. New York: Plenum Press; 1983; 425-444.

Caroline, L; Kozinn, P. J.; Feldman, F; Stiefel, F. H. Infection and iron overload in thalassemia. Annals of New York Academy of Science 165:148-155; 1969.

Corbin, J. L.; Bulen, W. A. The isolation and identification of 2,3-dihydroxybenzoic acid and 2-N,G-N-Di-(2,3-dihydroxybenzoyl)-L-lysine formed by iron-deficient Azotobacter vinelandii. Biochemistry 8:757-762; 1969.

Coughlan, M. P. The role of iron in microbial metabolism. Sci. Prog. Oxf. 59:1-23; 1971.

Dautry-Varsat, A.; Lodish, H. F. How receptors bring proteins and particles into cells. Scientific American 250(5):52-58; 1984.

David, C. N. Ferritin and iron metabolism in Phycomyces. Neilands, J. B., ed. Microbial Iron Metabolism. New York: Academic Press; 1974; 149-158.

David, C. N.; Easterbrook, K. Ferritin in the fungus Phycomyces. The Journal of Cell Biology 48:15-28; 1971.

Despommier, D. D. Biology. Campbell, W. C., ed. Trichinella and Trichinellosis. New York: Plenum Press; 1983; 75-151.

Diamond, L. S.; Harlow, D. R.; Phillips, B. P.; Keister, D. B. Entamoeba histolytica: Iron and nutritional immunity. Arch. Invest. Méd. (Méx.). 9(Suppl. 1):329; 1978.

Emery, T. Isolation, characterization, and properties of fusarinine, a δ-hydroxamic acid derivative of ornithine. Biochemistry 4:1410; 1965.

Ferguson, J. D.; Castro, G. A. Metabolism of intestinal stages of Trichinella spiralis. Am. J. Physiol. 225:85-89; 1973.

Finkelstein R. A.; Yancey, R. J. Effect of siderophores on virulence of neisseria gonorrhoeae. Infection and Immunity 32:609-613; 1981.

Finkelstein, R. A.; Sciortino, C. V.; McIntosh, M. A. Role of iron in microbe-host interactions. Review of Infectious Diseases 5(Suppl. 4): 459-777; 1983.

Foster, L. A.; Bogitsh, B. J. Utilization of heme and its precursur aminolaevulinic acid by Schistosoma mansoni in vitro. AMCOP XXXVI, Annual Midwestern Conference of Parasitologists, Abstract 12a. Iowa City, June 7-9, 1984.

Francis, J.; Macturk, H. M.; Madinaveitia, J.; Snow, G. A. Mycobactin, a growth factor for Mycobacterium johnei. Biochem. J. 55:596-607; 1953.

Francis, J.; Madinaveitia, J.; Macturk, H. M.; Snow, G. A. Isolation from acid-fast bacteria of a growth-factor for Mycobacterium johnei and of a precursor of phthiocol. Nature 163:365-366; 1949.

Gameel, A. A. Fasciela hepatica: Plasma ascorbic acid, plasma iron and iron-binding capacity in experimentally infected sheep. Z. Parasitenkd. 68:185-189; 1982.

Gardiner, C. H. Habitat and reproductive behavior of Trichinella spiralis. J. Parasitol. 62:865-870; 1976.

Giblett, E. R.; Hickman, C. G; Smithies, O. Serum transferrins. Nature 183:1589-1590; 1959.

Gladstone, G. P. The effect of iron and haematin on the killing of staphylococci by rabbit polymorphonuclear leucocytes. Contribution to Microbiology. Vol. 1. Staph infections. Basel: Karger; 1973; 222-243.

Griffiths, E. Bacterial adaptation to low-iron environment. Schlessinger, D., ed. Microbiology-1983. Washington, D.C.: American Society for Microbiology; 1983; 329-303.

Harley, J. P.; Gallicchio, V. Trichinella spiralis: Migration of larvae in the rat. Exp. Parasitol. 30:11-12; 1971.

Harrison, P. M.; Hoare, R. J.; Hoy, T. G.; Macara, I. G. Ferritin and haemosiderin: Structure and function. Jacobs, A., Worwood, M., eds. Iron in Biochemistry and Medicine. New York: Academic Press; 1974; 73-114.

Jones, R. L.; Peterson, C. M.; Grady, R. W.; Cerami, A. Low molecular weight iron-binding factor from mammalian tissue that potentiates bacterial growth. J. Exp. Med. 151:418-428; 1980.

Kluger, M. J.; Rothenburg, B. A. Fever and reduced iron: Their interaction as a host defense response to bacterial infection. Science 203:374-376; 1979.

Kluger, M. J.; Tocco, R. J.; Ucar, D. A. Physiological aspects of fever and iron metabolism. Schlessinger, D., ed. Microbiology-1983. Washington, D.C.: American Society for Microbiology; 1983; 338-341.

Kochan, I. Nutritional regulation of antibacterial resistance. Schlessinger, D., ed. Microbiology-1974. Washington, D.C.: American Society for Microbiology; 1974; 273-288.

Kochan, I. Role of iron in regulation of nutritional immunity. Adv. Chem. Ser. 162:55-77; 1977.

Kochan, I. Neutralization of acquired antibacterial immunity with iron. Schlessinger, D., ed. Microbiology-1983. Washington, D.C.: American Society for Microbiology; 1983; 342-345.

Kochan, I.; Wagner, S. K. Effect of iron on the antibody-dependent immunity to Salmonella typhimurium. Abstracts of the Annual Meeting-1984: Abstract B116. American Society for Microbiology. 84th Annual Meeting, St. Louis, Missouri, 4-9 Mar. 1984.

Kochan, I.; Wasynczuk, J.; McCabe, M. A. Effects of injected iron and siderophores on infections in normal and immune mice. Infection and Immunity 22:560-567; 1978.

Konijn, A. M.; Hershko, C. Ferritin synthesis in inflammation. I. Pathogenesis of Impaired Iron Release. Br. J. Haemotol. 37:7-16; 1977.

Lankford, C. E. Bacterial assimilation of iron. C.R.C. Critical Rev. Microb. 2:273-331; 1973.

Latour, N. G.; Reeves, R. E. An iron-requirement for growth of Entamoeba histolytica in culture, and the antamebal activity of 7-iodo-8-bydroxy-guineline-5-sulfuric acid. Exp. Parasitol. 17:203; 1965.

Lee, C. M.; George, Y. G.; Cole, A. Iron metabolism in Trypanosoma lewisi infection: Serum iron and serum iron-binding capacity. Z. Parasitenkd. 53:1-6; 1977a.

Lee, C. M.; George, Y. G.; Cole, G. F. A. Interaction of nutrition and infection: Effect of iron deficiency on resistance to Trypanosoma lewisi. Int. J. Biochem. 8:525-529; 1977b.

Letendre, E. D.; Holbein, B. E. Turnover in the transferrin iron pool during the hypoferremic phase of experimental Neisseria meningitidis infection in mice. Infection and Immunity 39:50-59; 1983.

Locke, A; Main, E. R.; Rosbash, D. O. The copper and non-hemoglobinous iron contents of the blood serum in disease. J. Clinic Investig. 11: 527-542; 1932.

Macleod, R. A.; Snell, E. E. Some mineral requirements of the lactic acid bacteria. J. Biol. Chem. 170:351-365; 1947.

Masson, P. L.; Heremans, J. F.; Dive, C. H. An iron-binding protein common to many external secretions. Clinica Chimica Acta 14:735-739; 1966.

Masson, P. L.; Heremans, J. F.; Schonne, E. Lactoferrin an iron-binding protein in neutrophilic leucocytes. J. Exp. Medicine 130:643; 1969.

Meerovitch, E. Studies on the in vitro axenic development of Trichinella spiralis. I. Basic Culture Techniques, Pattern of Development, and the Effects of the Gaseous Phase. Canadian Journal of Zoology 43:69-79; 1965.

Mietzner, T.; Luginbuhl, G.; and Morse, S. A. A new major iron-regulated outer membrane protein of Neisseria gonorrhoeae. Abstracts of the Annual Meeting-1984; Abstract D13. American Society for Microbiology. 84th Annual Meeting, St. Louis, Missouri, 4-9 Mar. 1984.

Moore, D. G.; Yancey, R. G.; Lankford, C. E.; Earhart, C. F. Bacteriostatic enterochelin-specific immunoglobulin from normal human serum. Infection and Immunity 27:418-423; 1980.

Morgan, E. H. Transferrin and Transferrin iron. Jacobs, A. and Wozwood, M., ed. Iron in Biochemistry and Medicine. New York: Academic Press; 1974; 29-71.

Murray, M. J.; Murray, A. B. Starvation suppression and refeeding activation of infection. An ecological necessity? Lancet i:123-125; 1977a.

Murray, J.; Murray, A. Suppression of infection by famine and its activation by refeeding -- a paradox? Perspectives in Biology and Medicine 20:471-483; 1977b.

Murray, M. J.; Murray, A. B.; Murray, N. J.; Murray, M. B. (Intr. by Butt, H.). Cerebral malaria, hyperferremia and folate deficiency. Clinical Res. 23:457A; 1975a.

Murray, M. J.; Murray, N. J.; Murray, A. B.; Murray, M. B. Refeeding-malaria and hyperferremia. Lancet i:653-654; 1975b.

Murray, M. J.; Murray, M. B.; Murray, A. B.; Murray, C. J. Somali food shelters in the Ogaden famine and their impact on health. Lancet i: 1283-1285; 1976.

Murray, M. J.; Murray, A.; Murray, C. J. The salutary effect of milk on amoebiasis and its reversal by iron. British Medical Journal 280 (6228):1351-1352; 1980.

Nafie, T.; El-Allawy, T.; Mottelib, A. A.; El-Amrousi, S. Effect of induced equine piroplasmosis on some blood constituents. Assiut Veterinary Medical Journal 9:123-128; 1982.

Neilands, J. B. Some aspects of microbial iron metabolism. Bacteriological Reviews 21:101-111; 1957.

Neilands, J. B. Evolution of biological iron binding centers. Struct. Bonding, Berlin 11:145-170; 1972.

Neilands, J. B. Microbial iron compounds. Ann. Rev. Biochem. 50:715-731; 1981.

O'Brien, J. G.; Gibson, F. The structure of enterochelin and related 2,3-dihydroxy-N-benzoylserine conjugates from Escherichia coli. Biochimica et Biophysica Acta 215:393-402; 1970.

Olsen, B. S.; Villela, J. B.; Gould, S. E. Distribution of Trichinella spiralis in muscles of experimentally infected swine. J. Parasitol. 50:489-495; 1964.

Ong, S. A.; Peterson, T.; Neilands, J. B. Agrobactin, a siderophore from Agrobacterium tumefaciens. The Journal of Biological Chemistry 254:1860-1865; 1979.

Oseas, R.; Yang, H. H.; Baehner, R. L.; Boxer, L. A. Lactoferrin: A promoter of polymorphonuclear leucocyte adhesiveness. Blood 57:939-945; 1981.

Payne, M. S. Siderophores and acquisition of iron by Gram neg. pathogens. Schlessinger, D., ed. Microbiology-1983. Washington, D.C.: American Society for Microbiology; 1983; 346-349.

Pekarek, R. S.; Wannemacher, R. W.; Beisel, J.; Beisel, W. R. The effect of leukocytic endogenous mediator (LEM) on the tissue distribution of zinc and iron. Proceedings Soc. Exp. Biol. Med. 140:685-688; 1972.

Perry, R. D; Brubaker, R. R. Role of pigmentation in iron and hemin storage by Yersinia pestis. Abstracts of the Annual Meeting-1984: Abstract D58. American Society for Microbiology. 84th Annual Meeting. St. Louis, Missouri, 4-9 Mar. 1984.

Piercy, D. W. T. Acute phase responses to experimental salmonellosis in calves and colibacillosis in chickens: Serum iron and caeruloplasmin. J. Comp. Pathol. 89:309-319; 1979.

Pollack, J. R.; Neilands, J. B. Enterobactin, an iron transport compound from Salmonella typhimurium. Biochemical and Biophysical Research Communications 38:989-992; 1970.

Pryzwansky, K. B.; Martin, L. E.; Spitznagel, J. K. Immunocytochemical localization of myeloperoxides, lactoferrin, lysozyme and neutral proteases in human monocytes and neutrophilic granulocytes. J. Reticuloendothelial Soc. 24:295; 1978.

Puschmann, M.; Ganzoni, A. M. Increased resistance of iron-deficient mice to Salmonella infection. Infection and Immunity 17:663-664; 1977.

Raventos-Suarez, C.; Pollack, S.; Nagel, R. L. Plasmodium falciparum: Inhibition of in vitro growth by desferrioxamine. Am. J. Trop. Med. Hyg. 31:919-922; 1982.

Reiter, B.; Oram, J. D. Bacterial inhibitors in milk and other biological fluids. Nature 216:328; 1967.

Rogers, H. J. Iron-binding catechols and virulence in Escherichia coli. Infect. Immunity 7:445-456; 1973.

Rogers, H. J. Role of iron chelators, antibodies, and iron-binding proteins in infection. Schlessinger, D., ed. Microbiology-1983. Washington, D.C.: American Society for Microbiology; 1983; 334-337.

Rogers, H. J.; Synge, C. Bacteriostatic effect of human milk on Escherichia coli: The role of IgA. Immunology 34:19-28; 1978.

Schade, A. L. Growth of Staphylococcus aureus as controlled by the percentage of iron saturation of the ekkrino-siderophilin (lactoferrin) of human milk. Schlessinger, D., ed. Microbiology-1974. Washington, D.C.: American Society for Microbiology; 1974; 266-269.

Schade, A. L.; Caroline, L. Raw hen egg white and the role of iron in growth inhibition of Shigella dysenteriae, Staphylococcus aureus, Escherichia coli and Saecharomyces cerevisiae. Science 100:14-15; 1944.

Schade, A. L.; Caroline, L. An iron-binding component in human blood plasma. Science 104:340-341; 1946.

Simonson, C.; DeVoe, I. W. Removal of iron from transferrin at the meningococcal surface. Schlessinger, D., ed. Microbiology-1983. Washington, D.C.: American Society for Microbiology; 1983; 350-353.

Simpson, L. M.; Wright, A. C.; Oliver, J. D. Influence of iron on infections produced by clinical and environmental strains of Vibrio vulnificus. Abstracts of the Annual Meeting-1984: Abstract B133. American Society for Microbiology. 84th Annual Meeting, St. Louis, Missouri, 4-9 Mar. 1984.

Sinclair, K. B. Iron metabolism in ovine fascioliasis. Brit. Vet. J. 121:451-461; 1965.

Sismeev, V. P. The influence of Trichinella infection on the trace elements in the spleen of white rats. Problemy Parazitologii. Materialy VIII Nauchnoi Konferentsii Parazitologov Uk SSR. Chast' 2. Kiev, USSR; "Naukova Dumka"; 1975; 166-167.

Smith, K. L.; Schanbacher, F. L. Lactoferrin as a factor of resistance to infection of the bovine mammary gland. JAVMA 170:1224-1227; 1977.

Snow, G. A. Mycobactins: Iron-chelating growth factors from mycobacteria. Bacteriological Reviews 34:99-152; 1970.

Sousa, M. Iron and lymphomyeloid system: Old frontier, new perspective. Schlessinger, D., ed. Microbiology-1983. Washington, D.C.: American Society for Microbiology; 1983; 322-326.

Spiro, T. G.; Saltman, P. Polynuclear complexes of iron and their biological implications. Struct. Bonding, Berlin 6:116-156; 1969.

Spiro, T. G.; Saltman, P. Inorganic chemistry. Jacobs, A. and Worwood, M., eds. Iron in biochemistry and medicine. New York: Academic Press; 1974; 1-28.

Stahr, H. M., ed. Analytical Toxicology Methods Manual. Iowa State University Press, Ames, Iowa; 1977; 47.

Stewart, G. L. Studies on biochemical pathology in trichinosis. II. Changes in liver and muscle glycogen and some blood chemical parameters in mice. Rice University Studies 62:211-224; 1976.

Stewart, G. L. Biochemistry. Campbell, W. C., ed. Trichinella and trichinellosis. New York: Plenum Press; 1983; 153-172.

Stiefel, E. I; Watt, G. D. Azotobacter cytochrome $b_{557.5}$ is a bacterioferritin. Nature 279:81-83; 1979.

Surgenor, D. M.; Koechlin, B. A.; Strong, L. E. Chemical, clinical and immunological studies on the products of human plasma fractionation XXXVII. The Metal Combining Globulin of Human Plasma. J. Clin. Invest. 28:73-78; 1949.

Tait, G. H. The identification and biosynthesis of siderochromes formed by Micrococcus denitrificans. Biochem. J. 146:191-204; 1975.

Tartour, G.; Idris, O. F. Iron metabolism in Trypanasoma congolense infection in Zebu cattle: Serum iron and serum iron-binding capacity. Res. Vet. Sci. 15:24-32; 1973.

Teuwissen, B.; Masson, P. L.; Osinski, P.; Heremans, J. F. Metal combining properties of human lactoferrin. The possible involvement of tyrosyl residues in the binding sites. Spectrophotometric titration. Eur. J. Biochem. 31:239-245; 1972.

Van Snick, J. L.; Masson, P. L.; Heremans, J. F. The involvement of lactoferrin in the hyposideremia of acute inflammation. J. Exp. Medicine 140:1068-1084; 1974.

Victor, O. A.; Kaneko, J. J. Pathogenesis of Trypanosoma brucei infection in deer mice (Peromyscus maniculatus): Hematologic, erythrocyte, biochemical, and iron metabolic aspects. Am. J. Vet. Res. 44:639-644; 1983.

Vieira, R. A. O ferro sanguineo em cães com ancilostomiase experimental. An. do Instituto de Hygiene e Med. Trop. (Lisboa) 1:439-450; 1974.

Weinberg, E. D. Iron and susceptibility to infectious disease. Science 184:952-956; 1974.

Weinberg, E. D. Iron and infection. Microbiological Reviews 42:45-66; 1978.

West, S. E. H.; Blackman, E.; Sparling, P. F. Iron-repressible membrane proteins from Neisseria gonorrhoeae. Abstracts of the Annual Meeting-1984: Abstract B121. American Society for Microbiology. 84th Annual Meeting, St. Louis, Missouri, 4-9 Mar. 1984.

Wright, K. Trichinella spiralis: An intracellular parasite in the intestinal phase. J. Parasitol. 65:441-445; 1979.

Zajic, J. E. Microbial biogeochemistry. New York: Academic Press; 1969.

Zimmermann, W. J.; Schwarte, L. H. Distribution of Trichinella spiralis larvae in tissues of swine. Iowa Academy of Science 68:553-557; 1961.

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